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THE CHANGES IN THE BLOOD SUGAR WHICH FOLLOW EXPERIMENTAL THYROID FEEDING

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There are available for therapeutic purposes a great number of thyroid preparations. Each seems to be made by a different method and their clinical effects are far from constant. The variables are probably due in part to the personal idiosyncrasies or peculiarities of the individual's pathological physiology, and in part to the medicament. For some represent the whole gland substance which has apparently been dried at a high or low temperature, with or without "defatting," and others are chemical derivatives or extracts of the organ. We have attempted to devise some method of standardization which would show more or less accurately the physiological activity of the different forms of this widely used medicament.

After some preliminary studies, we investigated the changes in the sugar content of the blood[1] following the oral administration to dogs of four of the most widely advertised and most commonly used commercial preparations; their types can be characterized as (P) "dried" (U. S. P.): as (S) high in their nucleoprotein content: as (B) high in globulin content: as (T) high in thyroxin content. We have compared these with a glycerol extract of the fresh gland. A glycerol extract, by the way, seems to have been the first method employed to escape prescribing the hashed raw gland. It possesses many advantages:

1. The pure glycerol, because of its power for absorbing water, breaks up the cell organization of the finely ground thyroid and sets free the cell content without material chemical change in its freed constituents.

[1] It may be recalled that in diabetes the glycosuria is always intensified by thyroid feeding.

2. The corpuscles in the blood of the fresh glands furnish an apparently protective, adsorptive surface; for it was found that the addition of a small amount of fresh defibrinated beef blood increased the iodin content of the extract between 7 and 10 per cent.

3. Then after filtration it was found that washing the gland residue on the filter with distilled water gave an additional 10 per cent of iodin in the finished filtrate.

4. The extract completed by this washing was then found to contain from 85 to 90 per cent of the total iodin in the fresh glands, and the total volume of this completed extract could be thus made to weigh approximately five times the weight of the original fresh glands.

5. The water in the mixture was then evaporated at 37°C., but after some experimentation the resultant concentrated glycerol extract was found, in the course of days or weeks of exposure to sunlight and room temperature, to change in appearance and to lose some of its activity.

6. Therefore, the concentrated glycerol extract was mixed with several substances to produce a solid.

7. We finally found that any one of several inert vegetable powders would enable the solid mass to be made into large pills which could be gelatin-coated, and in this form retain more or less indefinitely an "activity" as great as a fresh glycerol extract according to the blood sugar determinations.

This matter of the deterioration of a glycerol extract is of great practical importance, both in the therapeutic use of this material and in its laboratory tests upon animals which may require weeks or months of observation.

Experimental procedure. The four commercial thyroid preparations which were selected because they seemed to be most commonly prescribed, and this glycerol extract, were analyzed for their iodin content by Kendall's method,[2] and at a later time these analyses were again made and checked by a different worker.

1. The approximate normal average blood sugar curve was established on six dogs that were accustomed to being bled. The samples were taken either from the femoral or great saphenous veins at ½-hour, 1-hour, 2½-hour and 4-hour intervals, and the blood sugar estimated by the Benedict procedure. In every case the dogs were fed a constant diet at approximately the same hour as the preceding day.

2. A single dose of glycerol mixed with blood in the same proportions as is found in the glycerol extract of thyroid was given by mouth to six dogs and the blood sugar determined at the same intervals.

3. Amounts of the five preparations were given in single doses by mouth, such that each dog received an equi-molecular quantity of iodin, as based on the analysis.

[2] Journ. Biol. Chem., 1920, xliii, 149.

4. The preparations were given at intervals of two weeks in order to avoid any lasting effect of the previous dose.

5. Two series of dogs were given single doses of the same preparation on the same day at 2-week intervals throughout the experiment, and in a third series, each dog received a single different preparation on the same day at 2-week intervals. The matter of succession of preparations made no demonstrable difference in the average blood sugar curve.

6. The glycerol extract in pill form was given at the close of the experiment to some of the dogs, while a comparable fresh glycerol extract was given to another group.

No diminution of the activity of the glycerol extract of thyroid was noted as a result of the preceding large dosage of any commercial thyroid preparations, at 2-week intervals, nor were the gelatin-coated pills made from the glycerol thyroid mass any less efficient than the fresh (liquid) glycerol extract.

The equivalent amounts given according to their iodin content were:

5 cc. glycerol extract thyroid
2.25 of the 5 grain commercial tablets (B) (rich in globulin)
36 one grain commercial tablets (S) (rich in nucleoproteins)
2.75 of the 2 grain commercial tablets (P) (dried: U S P)
½ or 0.5 of the 2 grain commercial tablets (T) (rich in thyroxin)

Each of these materials contained 0.72 mgm. of iodine, or about fifteen times the average human dose.

The curves shown in the accompanying chart will best represent the comparative effects of the different preparations upon the blood sugar. They show the composite average of the rise or fall of blood sugar in milligrams per 100 cc. above or below the normal average at the stated time intervals. This chart is made from about four hundred blood sugar determinations.

Discussion. It will be noted that the normal or control dogs which received no treatment showed a rise in blood sugar which was as great or greater than the dogs which were fed the thyroid preparations. This is probably due to the freedom and unusual activity of the animal just before drawing the blood. Previously, they had been separately confined in cages. But in the morning of the day they were bled they were liberated in the operating room and had an opportunity for unrestrained exercise.

The strikingly significant fact brought out by these curves is the maintained rise in blood sugar level above the normal produced by the single oral administration of the glycerol extract of thyroid. It is not maintained by the like administration of any of the four commercial products.

We have in preparation an article on the basal metabolic rate following the administration of these materials, and the findings seem to correspond

quite closely with the blood sugar determinations. It can be stated in advance that the glycerol extract of thyroid apparently produces an average elevation of 15 per cent above the others.

In the preliminary studies an attempt was made to administer at intervals of a week the glycerol extract of thyroid intravenously, but the protein sensitization or anaphylactic reaction seemed to confuse the results. This effect was not noticeable when the injection interval was reduced to two days. Then the rapidity and amplitude of the rise in blood sugar was

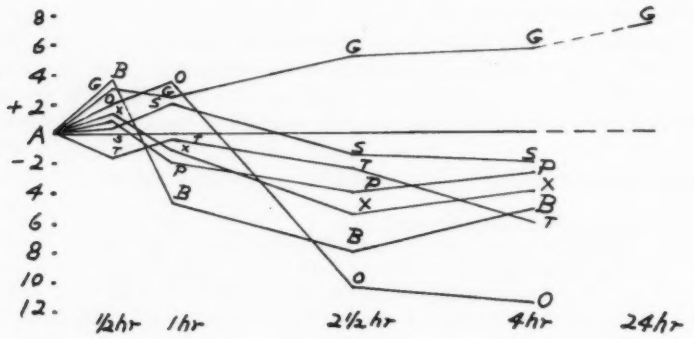

Fig. 1

A = average normal blood sugar.
\+ = rise above normal in milligrams per 100 cc.
− = fall below normal in milligrams per 100 cc.
(*G*) = glycerol thyroid.
G' = 24 hour rise on 3 dogs only.
O = Normal blood sugar.
X = glycerol blood.
B, S, P and T = the four commercial preparations.

much greater with much smaller quantities of the glycerol extract when it was given intravenously than when given by mouth.

The following is a condensed résumé of this study:

INJECTION INTERVAL	NUMBER OF DOGS	AMOUNT OF GLYCERIN EXTRACT THYROID	AVERAGE 1-HOUR CHANGE (GAIN)	AVERAGE 4-HOUR CHANGE (FALL)
		cc.	*mgm.*	*mgm.*
1 week	23	4	+21.5	−7.5
2 days	9	3	+8.0	+3.0

The intravenous use of a glycerol extract of thyroid is, however, not generally practicable.

SUMMARY

The oral administration to dogs of a glycerol extract of hashed fresh (pig) thyroid glands produces a marked and sustained rise in the sugar content of the animal's blood.

This is not shown by any of four commonly used commercial preparations of the medicament. One of these, in fact, shows an immediate and distinct decrease in the blood sugar.

We wish to express our appreciation of the skilful and unremitting care of these animals by Rudolph Himmel.

THE EFFECT OF POTASSIUM AND CALCIUM ON THE RESPONSE OF THE ISOLATED FROG HEART TO NICOTINE

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Various observers have studied the action of nicotine on the heart of different animals. Truhart (1) was apparently the first to report an investigation on its effect on the frog heart which he made on the intact animal. Later, Beyer (2) carried out experiments on the isolated heart of the cat, and, within recent years, studies have been made by Fleig on the effect of nicotine on the isolated heart of different mammals (3), but particularly noteworthy are the studies made by Clerc and Pezzi (4). To Hett (5) belongs the credit of making the first attempt to determine its action on the isolated frog heart. These studies, it may be observed, also included experiments on the effect of Ca, Mg and adrenalin on the reaction of the heart to nicotine. As the results he obtained are of particular interest in connection with our own investigation, a summary of his findings is presented. Hett stated that in experiments in which the intracardiac vagus mechanism was intact, concentrations varying between 1–200,000 to 1–20,000 decreased the force and frequency of the concentrations, produced cardiac irregularity and sometimes caused prolonged arrest of the heart which lasted 18 seconds to 10 minutes. No inhibition occurred, however, when the concentration exceeded 1–2,500. The effect, according to Hett, varied also with the concentration of nicotine when the vagal terminations were paralyzed by atropine. The amplitude was increased by nicotine in a dilution of 1–100,000 without causing arrest of the heart, while stronger concentrations sometimes stimulated, and sometimes depressed the heart and also caused prolongation of the A-V interval.

The object of our investigation was mainly to determine the effect of potassium and calcium on the reaction of the frog heart to nicotine. But we soon found that a re-investigation of the action of nicotine alone was also necessary. The experiments were carried out by the methods employed in a previous investigation published from this laboratory (6). The composition of the solutions was as follows.

Normal Ringer (R 2)

NaCl	0.65 per cent
KCl	0.014 per cent

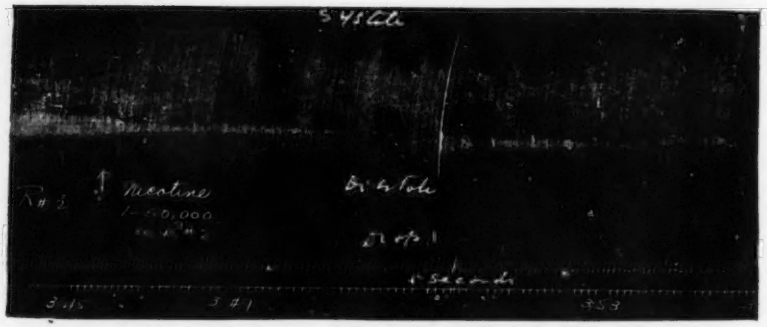
$CaCl$	0.012 per cent
$NaHCO_3$	0.02 per cent
NaH_2PO_4	0.001 per cent

pH 7.4

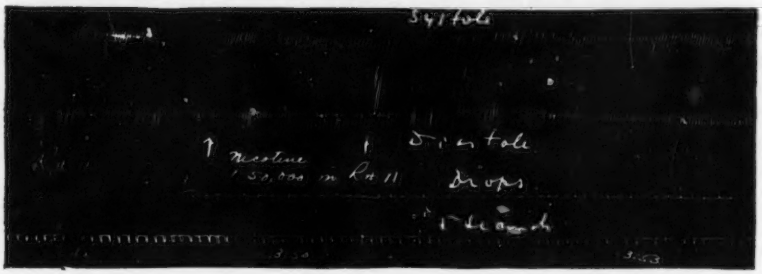
R 7 Ringer with 0.036 per cent $CaCl_2$
R 12b Ringer with 0.006 per cent $CaCl_2$
R 11 Ringer with 0.056 per cent KCl
R 10 Ringer with 0.028 per cent KCl

EXPERIMENTS WITH NICOTINE IN NORMAL RINGER'S SOLUTION. Of the various changes produced by nicotine, those observed after perfusing the heart with dilute solutions, 1–100,000 and 1–1,000,000 may be considered first. In the fresh heart with regular and fairly vigorous contractions, weak solutions caused stimulation, and it may be interesting to observe that the stimulating action was greater after perfusion with nicotine in 1–1,000,000 than in 1–100,000. In about one minute the force of the beat was increased, the amplitude of the contractions showing, in some cases, an augmentation of about 30 per cent which was due to a more vigorous systolic contraction as well as to more complete relaxation. Exceptionally, however, cardiac depression was also observed, but this occurred only when the heart was perfused with nicotine without preliminary treatment with atropine. The initial effect was a moderate and transitory decrease in the force of the contractions, but no change in rate occurred when the vagus mechanism was intact. That nicotine in small amounts was a cardiac stimulant was demonstrated even more satisfactorily in a series of experiments in which the heart had been depressed by toxic doses of atropine sulphate. The amplitude was lengthened about 50 per cent, the systole, and even to a greater extent the relaxation in diastole, being increased. No very great change could be observed, however, in the speed of the heart, but an increase of 8 to 10 per cent in the frequency of the contraction sometimes occurred.

The results were different if the amount of nicotine in the perfusate was increased to 1–50,000. In a few experiments no change occurred, but in most of them a moderate decrease in the force of the beat was observed, while in three experiments the depression of the heart was considerable, the amplitude of the contractions being decreased 30 to 60 per cent. The heart in one of these, it may be observed, was very weak, which leaves only two experiments in which a medium concentration of nicotine caused a very pronounced depression. But these were apparently atypical cases for in a larger number of experiments with this concentration of nicotine, carried out under a variety of conditions, such extensive depression was never observed. Indeed, even strong solutions seldom caused such a decrease in the force of the heart. Although a change in the rate of the



A



B

C

Fig. 1. *A*. Experiment 324 showing effect of nicotine 1–50,000 in normal Ringer's solution.

B. Experiment 303. The same concentration of nicotine as in *A*, but Ringer's solution contained four times as much potassium as in *A*. Shows that nicotine stimulated the intracardiac vagus ganglia when potassium was increased.

C. Experiment 39. Nicotine 1–1,000,000 in normal Ringer's solution stimulated the heart.

No atropine was used in these experiments before perfusion with nicotine.

contractions was rarely noticeable, and was very small when the vagus mechanism was intact, well-marked acceleration was produced by nicotine after treatment with atropine, the frequency of the contractions in one experiment being increased 35 per cent.

We also made observations on the effect of repeated perfusion with nicotine. As will be shown in experiments with strong solutions, nicotine was increasingly effective after successive perfusions, but this was never observed with weak and medium solutions. Only a few experiments were made with nicotine in a concentration of 1–10,000. The results were not much different from those obtained in the preceding experiments. The force as well as the frequency was reduced in one and no change was observed in the other experiments.

A more extended series of observations was made with 1–5000 nicotine. The effect was as follows: The amplitude of the contractions was decreased 40 per cent in one experiment after the heart had been perfused for 9 minutes; the rate was also decreased but not to the same extent. A considerable, though gradual, depression was also noticed in two others in which the duration of the perfusion lasted 16 to 17 minutes. But in several experiments, perfusions of the fresh heart for 3 to 9 minutes scarcely produced any change or moderately decreased the force of the contractions, while in some cases heart action became irregular, the contractions occurring in groups of two to five. When the heart had been previously perfused with atropine, the reaction to nicotine was generally the same as when the vagus mechanism was intact. Group contractions, however, occurred in a larger proportion of experiments when the heart was perfused with nicotine after atropine. We also noticed in these and in a number of other experiments that the initial effect was different, as immediately after the perfusion with nicotine was begun, a distinct, though small, increase in the force of the contraction was observed. The response of the heart to repeated perfusion with 1–5000 nicotine showed that the toxicity was greatly increased with the number of exposures to the alkaloid.

Studies were also made on the response of the heart when the concentration of nicotine was increased to 1–1000. While in a few experiments the first perfusion caused only moderate depression, the effect in some of them showed that it was very toxic. The amplitude of the contractions was decreased 20 to 50 per cent, and the heart action was also much slower. Exceptionally no change in rate was noticed and in one experiment slowing was preceded by acceleration. These changes were observed when the heart was perfused with nicotine without previous treatment with atropine. The amplitude and frequency of the contractions were also decreased when the heart was perfused with nicotine after atropine. The effect observed in experiments without atropine was not due, therefore, to vagus stimulation, but must have been caused by the action of nicotine

on cardiac muscle. Repeated perfusion with nicotine, the duration of the perfusion being approximately the same, greatly increased the depression which became severe when it was repeated a number of times. The amplitude was reduced to one-third in one experiment and was even smaller in another, the frequency being likewise decreased, but not nearly to the same extent as the force of the contractions. In some experiments, however, reperfusion with nicotine slowed the heart at first without decreasing the amplitude, and after 10 to 15 seconds stopped the ventricle in diastole, while the auricles continued to beat for some time. Nevertheless even in the most severe form of poisoning, perfusion with pure Ringer's solution restored the activity of the heart, the contractions became regular and as forcible as before nicotine.

EXPERIMENTS WITH NICOTINE AND EXCESS POTASSIUM. Observations were made on the effect of nicotine on the heart when the amount of KCl was increased to 0.028 and 0.056 per cent, that is, two and four times the quantity present in normal Ringer's solution. No striking changes in heart action were produced by the mere alteration in the composition of the perfusate. There was usually some depression immediately after perfusion with the pure solution was changed to one with high potassium, but the effect was small, and was occasionally followed by stimulation. More prolonged exposure to greater amounts of potassium, however, decreased the force as well as the frequency of the contractions and caused irregularity. Atropine had no effect on the action of increased amounts of potassium. In some cases, however, slight stimulation was observed when the atropinized heart was perfused with Ringer's solution containing increased amounts of potassium. It may be stated that, in general, excess potassium failed to exert any significant influence on heart action when the vagus mechanism was intact, and was sometimes without effect when the vagal terminations were paralyzed by atropine. After perfusion with nicotine in such solutions, the following changes in heart action were observed. When the amount of potassium was increased fourfold, nicotine in a concentration of 1–50,000 caused moderate slowing or even diastolic standstill typical of vagus stimulation in experiments in which the heart had not been perfused with atropine nor previously exposed to the action of nicotine. In one experiment, however, the same dilution of nicotine greatly accelerated the heart and only slightly increased the force of the beat, but heart action in this case was irregular before. The behavior of the heart was different after repeated perfusion with nicotine. The heart usually became irregular, group contractions with unequal beats occurred in some experiments, and decreased frequency or force of the contractions was observed in others. If nicotine was preceded by atropine, no change in rate was noticed whether Ringer's solutions contained double or four times the amount of potassium.

The effect of increasing the amount of potassium was also studied in experiments with higher concentrations of nicotine. In a solution containing 0.056 per cent KCl, nicotine, in a concentration of 1–10,000, produced irregularity and slowing of the heart. But the influence which excess of potassium may exert was very strikingly shown in experiments with stronger solutions of nicotine. The toxic effect was greatly increased

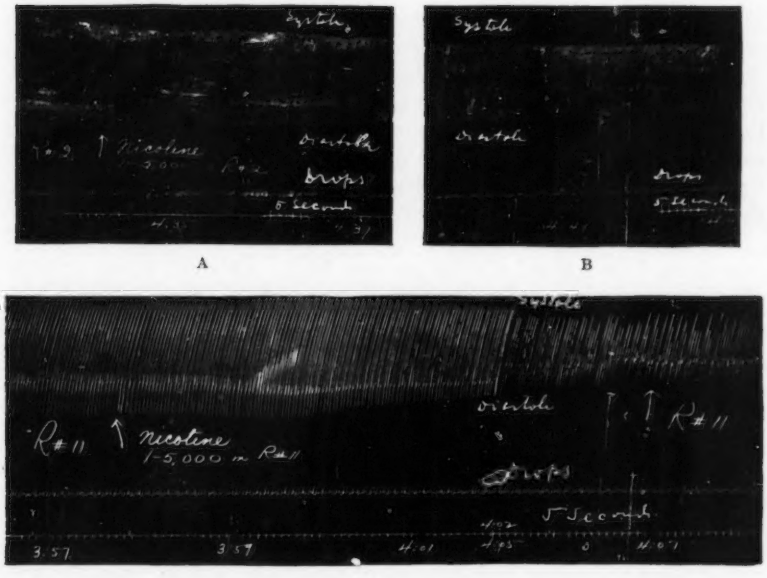


Fig. 2. Experiment 321. *A* and *B* show that nicotine 1–5,000 in normal Ringer's solution moderately decreased the force and frequency of the heart.

Experiment 336. *C*. The same concentration of nicotine as in experiment 321, but the amount of potassium was increased four times, and caused delirium cordis. Heart recovered when perfused with the same Ringer's without nicotine. No atropine was used before nicotine in either experiment.

when its concentration was doubled. Group contractions, heart block, which grew more intense with the duration of the perfusion, occurred in some experiments. Other signs of abnormal activity, such as decreased amplitude of the contractions at first, followed later by arrhythmia of the ventricles, were also observed. That the toxic effect was due to nicotine, and not to excessive amounts of potassium, was shown by the improvement which occurred when the heart was perfused with the pure solution. The injury which nicotine produced was more severe in other experiments, as

group contractions, unequal beats, dilatation, heart block, and, in some cases, delirium cordis were observed. In all cases, however, recovery occurred when nicotine was discontinued, which is particularly interesting in view of the statement made by Hett that when nicotine in concentrations of 1–20,000 and 1–10,000 produced standstill, washing of the heart caused a return of the contractions, but after higher concentrations, washing of the heart caused a further decrease in the force of the contractions. That the frog heart was very resistant to nicotine was also shown in these experiments, for decrease in the force of the contractions in two cases, and slowing in one of these, were the only effects observed. The results obtained after elimination of the vagus mechanism by atropine indicated that the reaction to nicotine when potassium was increased, was the same as when the vagal endings were intact. Observations on the effect of repeated perfusion with nicotine showed that, as in the other experiments, the toxicity was much increased by successive treatments with the drug.

Experiments were also carried out with nicotine in Ringer's solution containing 0.028 instead of 0.056 per cent KCl. Although it was without effect in one experiment, it proved to be very toxic in three others. The amplitude was decreased 20 per cent in one and 33 per cent in two others.

EXPERIMENTS WITH NICOTINE IN RINGER'S SOLUTION WITH DEFICIENT CALCIUM. Only three experiments were performed with nicotine in Ringer's solution containing half the normal amount of calcium, namely, 0.006 per cent $CaCl_2$. Nicotine 1–50,000 depressed the heart in every experiment. The amplitude of the contraction was decreased in one experiment 45 per cent within 2 minutes; in another the decrease was 33 per cent; while in the third it was nearly 40 per cent, which may be due to the slow rate of perfusion in this case. But the same rate of perfusion in the fore period failed to produce such decrease of amplitude; besides, when nicotine was discontinued, heart action improved. The speed of the heart was not affected in these experiments, though no atropine was used before, but it was noticed that the group contractions which developed in the fore-period, during perfusion with pure but modified Ringer's solution, disappeared upon the application of nicotine.

EXPERIMENTS WITH NICOTINE AND EXCESS CALCIUM. Increased amounts of calcium, 0.036 per cent, produced no recognizable change in the action of weak and medium concentrations of nicotine. The results were negative in all experiments whether the heart was perfused with a concentration of 1–500,000, 1–50,000 or even 1–10,000. Although the duration of the perfusion was, in most experiments, three to ten minutes, the results were the same when the period of exposure was sometimes prolonged to 26 minutes. Nor did the condition of the inhibitory apparatus exert any influence on the action of nicotine, for no effect was produced either when the vagus mechanism was intact nor when it was paralyzed by atropine.

Experiments were also carried out to determine the effect of an increased amount of calcium when the heart was perfused with strong concentrations of nicotine. The first test with a solution of 1–5000 produced no significant changes in three experiments and in one of these even a second perfusion was without any effect. Considerable depression was observed in a fourth experiment in which the rate was decreased 25 per cent, and the amplitude of the contractions was decreased 35 per cent, but this was very gradual as it was observed only at the end of 38 minutes. Besides, it was followed by a period of preliminary augmentation of the force of the contractions while the rate was decreased only 10 per cent. Well-marked symptoms of cardiac disturbances such as complete heart block and group contractions were observed in two other experiments, but this occurred in one of these after considerable stimulation by nicotine. The toxicity was, in all cases, augmented by reperfusion with the alkaloid, the effect being increased with each successive application of the drug. Heart block, extra systole, group contractions and cardiac dilatation developed in all but two experiments, and, in some cases, arrest of the auricles as well as of the ventricle, also occurred. The symptoms produced by nicotine were more severe when the heart had been perfused before with atropine sulphate.

In a few experiments, observations were also made with nicotine in a concentration of 1–1000. Dilatation occurred immediately after the alkaloid came in contact with the heart, but the contractions, though irregular, persisted. Notwithstanding the continued perfusion with nicotine, the ventricles ceased to contract only after an interval of 12 to 15 minutes, the auricles, however, continuing to beat. Especially noteworthy is the observation we made that reperfusion, though more effective, was not as injurious as with nicotine in normal Ringer's solution, and that higher tonus was sometimes also observed when the heart was perfused with a solution of 1–1000.

Discussion. It is apparent from the foregoing observations that the action of nicotine is subject to considerable variation depending upon its concentration and the composition of the perfusing medium. Small amounts of nicotine in normal Ringer's solution usually produced stimulation, while the same quantities in solutions containing an excess of calcium caused no effect. Again, medium concentrations were either inactive or slightly depressed the heart when the composition of the perfusate was unchanged, but produced a different effect when potassium or calcium was increased. Thus, a solution of 1–50,000 or even 1–10,000 nicotine in normal Ringer's solution sometimes failed to exert any influence on the heart or caused a moderate decrease of the amplitude with little or no change in rate. These concentrations in the presence of excess potassium greatly slowed the heart and even produced well-marked diastolic stand-

still. On the other hand, the same amount of nicotine was without any effect if calcium was increased even if the vagal terminations were not paralyzed by atropine. The results of our studies also showed that larger amounts of nicotine in normal Ringer's solution depressed the heart, causing a decrease of the amplitude as well as the rate of the contractions, and, besides, produced various forms of irregularity, all of which were greatly augmented by altering the composition of the perfusate. The changes in the effect of nicotine caused by potassium are especially worthy of remark, for not only was the heart more depressed than by perfusion with normal Ringer's solution and larger amounts of nicotine, but the symptoms of irregularity were more severe. Complete heart block was more frequent and delirium cordis was observed in these experiments only, which is particularly interesting in view of the results reported by Winterberg (7), who claimed that hyperexcitation of the vagus mechanism greatly facilitated auricular fibrillation produced by direct stimulation of the auricles. Since we observed delirium only in experiments in which the vagi had not been previously paralyzed by atropine, it may be assumed that increased sensitiveness was produced by potassium and this aided the effect of nicotine. Although greater concentrations of calcium in the perfusate likewise influenced the effect of nicotine, it is interesting to observe that the latter was not quite as toxic as in normal Ringer's solution, and was distinctly less active than in solutions with increased amounts of potassium. This suggests that cell permeability might have been a factor in the changes observed, for considerable evidence has accumulated of late indicating that potassium increases, and calcium decreases, cell permeability. The greater efficiency of medium amounts of nicotine in solutions containing only half the amount of calcium in normal Ringer's may be similarly accounted for as it has been shown that diminution of the ratio of Ca K is the important factor rather than the increase of the absolute amount of potassium (8). Although repeated perfusion with larger amounts of nicotine in Ringer's solution of different composition was highly toxic, the effect of the first exposure was quite different. We found in a large proportion of experiments that even high concentrations of nicotine (1–1000) often produced moderate depression only when the test on the heart was made for the first time. As this was carried out on the fresh heart, it may be assumed that heart muscle contains substances that neutralize various poisons, or that nicotine is cumulative. But, since little or no difference in the effect of repeated perfusion with medium and low concentrations of nicotine was observed, the decreased resistance which was produced by strong solutions, was probably caused by the loss of neutralizing substances which gains support from the work of Clark (9) who believed that the lipins were washed out of the heart by perfusion with Ringer's solution. The high resistance of the heart, as shown by its reaction to the first application of large amounts of nicotine as well as its

mild response when it was repeatedly perfused with low and medium concentrations, was also demonstrated by the fact that after severe symptoms had developed, perfusion with pure Ringer's solution restored the heart to regular and vigorous activity. It will have been noticed that our results differed in several respects from those which Hett obtained with nicotine on the frog heart. We never observed the long diastolic standstill which he reported. But his tables show that he found it only in 11 out of 39 experiments, and that he did not observe it when the concentration of nicotine was greater than 1–2500. He also reported that calcium was without effect. This, too, could not be corroborated by our experiments. As stated above, excess calcium does protect the heart against nicotine to a certain extent. However the conditions of his experiments and ours were not quite comparable. Most of our observation with nicotine lasted 10 to 15 minutes, while the duration, in some cases, was only 3 or 4 minutes, and in a few experiments only was perfusion with nicotine continued for any considerable length of time, namely, 30 to 38 minutes.

Our results as well as those of Hett indicate, however, that the action of nicotine on the frog heart differs from that observed in the mammalian heart. According to Beyer, nicotine greatly increased cardiac activity in the isolated heart of the cat. Fleig, and especially Clerc and Pezzi, showed that perfusion of the isolated heart of rabbits, dogs and monkeys with nicotine in concentration of about 1–20,000 caused a long diastolic standstill followed by a tremendous increase of the force and to a lesser extent also of the frequency of the contraction.

SUMMARY

1. Dilute concentrations of nicotine in normal Ringer's solution stimulated the frog heart; medium concentrations had no effect or caused depression. Strong solutions depressed the heart and also caused irregularity of action. The effect was not pronounced in some cases when the heart was perfused with strong solutions of nicotine for the first time, and was always increased by repeated perfusion. Evidence of vagus stimulation was obtained only with medium concentrations, but it was slight and not constant.

2. Excess of potassium in Ringer's solution greatly augmented the toxicity of nicotine and its inhibitory effect. Deficient calcium increased the depression produced by nicotine.

3. Excess of calcium in Ringer's solution diminished the effect of nicotine. The stimulation produced by dilute solutions was suppressed and the toxicity of strong solutions of nicotine was decreased.

4. That the frog heart was resistant to nicotine was shown by its mild reaction to the first perfusion with strong solutions and by the recovery from pronounced toxic effects when it was perfused with pure Ringer's solution.

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THE EFFECT OF THE CONCENTRATION OF HYDROGEN IONS ON THE ACTION OF NICOTINE

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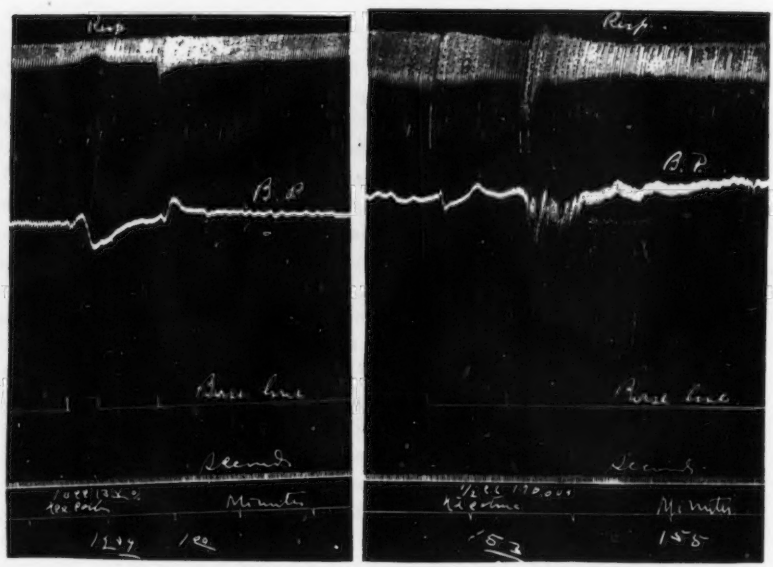
Since the early eighties of the last century, a number of reports have appeared dealing with the effect of the hydrogen ion concentration of the medium on the pharmacologic action of different substances which it held in solution. But, as shown in a review of the literature by Salant and Johnston (1), the scope of the studies was limited. Most of the experiments were made on the isolated heart; a few included observations on perfused blood vessels and only one investigation showed an attempt to determine the effect of a substance in vivo when the reaction of the circulating blood was changed.

Thus Collip (2) carried out experiments showing that the effect of the intravenous injection of small doses of epinephrin could be reversed by the administration of sufficient amounts of acid and alkali, the former decreasing, the latter increasing its pressor action. The observations of MacNider (3) may also be cited in this connection, as this investigator has shown that the response of the kidney to anesthetics is profoundly influenced by the acid base equilibrium of the tissues. The recognition of the importance of the reaction of biological fluids in determining the response to different substances, whether foreign or products of metabolic activity, prompted the writer to test the effect of a number of drugs when administered after acid and alkali, which, as established by several investigators, notably Levy, Rowntree and Marriott (4), Van Slyke and Cullen (5), Palmers and Van Slyke (6), Lumiere and Sors (7), caused considerable changes in the hydrogen ion concentration of the blood. The present report deals with a study of the pressor action of nicotine as affected by intravenous injections of hydrochloric acid, sodium acid phosphate and sodium carbonate.

METHODS. The experiments were carried out on cats and dogs. Urethane, usually with a small amount of ether, was employed for anesthesia, but in a few cats, ether alone was given. All the injections were made into the femoral vein. Nicotine was given in a concentration of 0.01 per cent, hydrochloric acid 1 per cent, sodium acid phosphate 1 and 2 per cent, sodium carbonate 1.34 to 3.5 per cent.

The action of nicotine was studied on animals with the vagi intact and also after the nerves had been cut. In some experiments this was followed by the injection of 0.01 mgm. atropine sulphate per kilo—a dose which was usually effective as found by the absence of reaction to faradic stimulation of the vagi nerves. The effect of acid and alkali on nicotine was also tested in cats from which the adrenals had been removed. Changes in volume of the kidney were studied by means of the oncometer. Blood pressure was recorded by a mercury manometer and respiration by a tambour connected with a metallic tube in the thoracic cavity.



A B

Fig. 1. Experiment 421. Cat, weight 2.5 kilos. Ether-urethane. Vagi intact. *A*, half cubic centimeter 1–10,000 nicotine injected 1 minute after 10 cc. 1.34 per cent sodium carbonate, caused slight pressor action without any effect on cardiac vagus mechanism. *B*, cardiac inhibition produced by the same amount of nicotine as in *A*, injected 1 minute after the intravenous administration of 30 cc. of 1 per cent HCl.

Results. Small amounts of nicotine—approximately 0.025 to 0.1 mgm. per kilo—injected intravenously into cats with vagi intact, produced cardiac inhibition, the effect differing, however, in intensity and duration with the size of the dose and also with the kind of anesthetic employed, both of which proved to be important factors in determining the vagus response. But when nicotine was given after the intravenous injection of sufficient amounts of acid sodium phosphate, or hydrochloric acid, its inhibitory

effect was greatly increased. There was a considerable drop in the blood pressure which sometimes lasted one minute instead of being of momentary duration, as was the case when no acid had been given previously. Quite different were the results when nicotine was administered after the injection of sodium carbonate. Cardiac inhibition was greatly decreased, and, in some experiments, scarcely any reaction could be obtained, the vagus response to nicotine being nil or insignificant. Since it is believed that nicotine first stimulates, then depresses, the intracardiac vagus ganglia, experiments were carried out to determine its effect after the alternate injection of acid and alkali, and it was found that its action could thus be reversed at will.

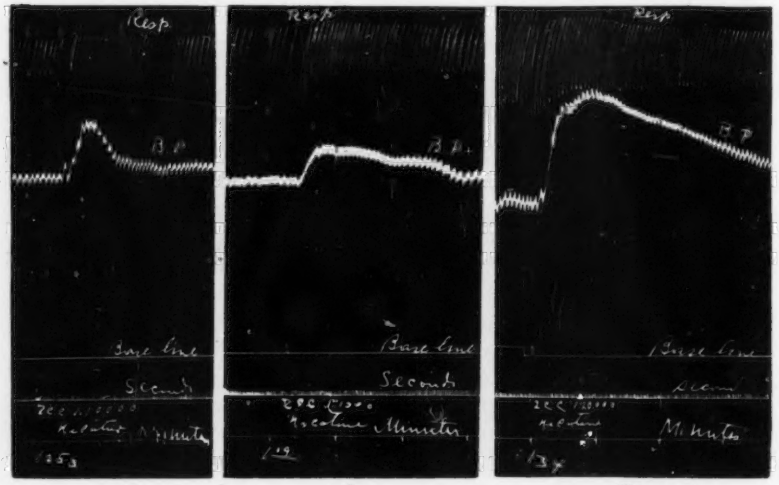
The results obtained in cats were corroborated later by experiments on dogs. Indeed, the vagus response to nicotine after acid was even more pronounced in these animals than in cats. A dose of 0.025 mgm. nicotine per kilo, administered after 15 cc. 1 per cent hydrochloric acid, was promptly followed in one experiment by the appearance of a very strong vagus pulse which, though diminished in intensity, lasted four minutes.

An entirely different picture was presented when, twenty minutes later, the same amount of nicotine was injected after 20 cc. of 3.3 per cent sodium carbonate. The vagus effect was much less than before, and was only of momentary duration, the heart regaining its rate within a few seconds. Similar results were obtained in the other three experiments on dogs. Indeed, in one of these, the carbonate completely abolished the reaction of the vagus mechanism to nicotine.

Experiments were also carried out on the effect of acid and alkali on the pressor action of nicotine. When it was injected intravenously into cats with vagi intact, a fall of blood pressure usually occurred which was soon followed by recovery, partial or complete, while in some cases the blood pressure not only regained its previous level, but even showed a considerable gain. When nicotine was injected shortly after a sufficient amount of acid was introduced into the blood stream, its pressor action was sometimes greatly increased, the rise of blood pressure being in some experiments in proportion to the amount of acid previously given. Thus, in one experiment, nicotine, injected after 10 cc. of 2 per cent sodium acid phosphate, increased the blood pressure 19 per cent, but the same amount of nicotine, given after double the quantity of acid, increased the blood pressure 45.5 per cent, and in one case the gain amounted to 163 and 180 per cent.

As the results described were obtained in cats under urethane anesthesia, only a small amount of ether being used at the beginning of the experiment, it was of interest to test the effect of the anesthetic on the reaction to nicotine, especially since it has been shown by Jackson and Ewing (8) that ether depressed the vagus mechanism. In two experiments in which

ether was used exclusively for producing anesthesia, the effect of acid and alkali proved to be closely similar to that observed when nicotine was administered to cats after the vagi had been divided, as cardiac inhibition and the accompanying fall of blood pressure were absent. The pressor action in one experiment was increased 42 per cent when nicotine was injected after acid, and ether was discontinued for a brief period. But the rise of blood pressure was increased 73 and 89 per cent when the same dose of nicotine after acid was given during ether inhalation. On the other hand, the pressor action of nicotine was greatly reduced by the previous



A B C

Fig. 2. Experiment 427. Cat, male, weight 3 kilos. Urethane. *A*, effect of the intravenous injection of 2 cc. of 1–10,000 nicotine, no acid or carbonate injected immediately before. *B*, the same dose of nicotine injected shortly after the intravenous administration of 20 cc. 1.34 per cent sodium carbonate. *C*, nicotine given as before, but injected after 20 cc. 1 per cent HCl. Both vagi were cut about 2 minutes before *A*.

administration of sodium carbonate, as the maximum rise of blood pressure did not exceed 31 per cent and sometimes amounted to 23 per cent even when it was injected during ether inhalation. Similar results were obtained in the other experiment, though the difference was less conspicuous. The rise of blood pressure produced by nicotine after acid varied between 30 and 38 per cent, but its effect after carbonate was nil, or was increased only 8 per cent, while nicotine alone increased the blood pressure 25 per cent. The carbonate, therefore, abolished the pressor action of nicotine also in ether anesthesia.

That the effect of nicotine on the blood pressure was augmented by acid and decreased by alkali was also observed in dogs with vagi intact, but the difference was less than in cats. In one experiment, however, the increase of blood pressure produced by nicotine after acid, amounted to 66 per cent, while the same dose given after carbonate raised it only 25 per cent.

It is evident, therefore, from the above observations, that alteration of the hydrogen ion concentration of the blood may produce considerable differences in the pressor action of nicotine in cats and also in dogs. But as these experiments were carried out on animals in which the vagus mechanism had not been previously disturbed, studies were also made on the behavior of nicotine if given after acid and alkali, when the vagi nerves had been cut and their endings paralyzed by atropine. The results obtained in these experiments with cats showed that, in cats, the difference in the effect of nicotine produced by varying the concentration of hydrogen ions, was greatly increased by the injection of acid. In a large number of injections the augmentation in the blood pressure, caused by nicotine after acid, was 50 to 70 per cent, and in two experiments it was 77 and 94 per cent. The increase in blood pressure when nicotine was given after carbonate was, in most cases, 14 to 30 per cent. In only two experiments was any considerable rise, 50 and 55 per cent, observed, but in two others the gain amounted to 7.5 and 9 per cent, which was less than after nicotine alone. Observations were also made on the circulatory changes when nicotine was injected after acid in vagotomized dogs. No effect was produced in three experiments, which was probably due to the large amounts of urethane necessary to induce and maintain anesthesia in these animals. In one experiment, however, 1 cc. 1–10,000 nicotine after acid, increased the blood pressure 30 and 60 per cent, whereas the same dose after sodium carbonate increased it only 12 and 30 per cent. The subject was a young dog weighing three kilos, and although it received 6.5 grams of urethane, respiration after double vagotomy was much better than in the other experiments on dogs, which may account for the difference in the effect produced. It is obvious, therefore, that a change in the reaction of the blood may modify the effect of nicotine in dogs, and more frequently in cats. The factors which may influence the response to nicotine in the body, described above, are illustrated by the following experiments.

Experiment 419. A cat weighing 2.1 kilos received 10 cc. ether by inhalation and 2.0 grams urethane intravenously. Injections of 1 cc. nicotine in a dilution of 1–10,000 were then made into the femoral vein. When the vagus mechanism was intact, nicotine alone increased the blood pressure 20 and 40 per cent, moderately stimulated inhibitory action also causing at the same time an increase in depth and frequency of respiration. Nicotine after acid increased the blood pressure 37.5 to 50 per cent, sometimes, but not always, provoked cardiac inhibition, and greatly stimulated respiration. When nicotine was given after carbonate, blood

pressure, which was lowered by the latter, was restored to the original level without changing the pulse rate. The effect on respiration was also less than that produced by nicotine after acid. An entirely different result was produced by nicotine when given after double vagotomy and paralysis of the vagal endings by atropine sulphate. When injected after acid, blood pressure was increased 93, 80 and 64 per cent, but after carbonate, the increase was only 27.7 and 36.8 per cent. The effect of nicotine on respiration was similar to that produced before division of the vagi.

Experiment 427. A cat, weighing 3 kilos, was given intravenously three grams urethane. Doses of 2 cc. 1–10,000 solution of nicotine (0.066 mgm. per kilo) were then administered at intervals of 16 to 26 minutes. When the vagi were intact, a prompt fall of blood pressure, associated with a large vagus pulse lasting about 20 seconds occurred. The blood pressure recovered, however, and gained about 15 per cent. The speed of the heart also improved, but was much slower than before. The injection of nicotine after 30 cc. 1.34 per cent carbonate, made 26 minutes later, was less effective. Within 24 minutes a third dose, which was given after 30 cc. 1 per cent hydrochloric acid, caused a greater response of the vagus mechanism and of the pressor reaction than the previous injections. When the injections of acid and nicotine were repeated 16 minutes later, cardiac inhibition was still more increased, the vagus pulse persisting for about 2 minutes.

The following changes in the circulation have been observed when nicotine was given after division of both vagi. Blood pressure rose from 160 to 200 mm. Hg or 25 per cent after the injection of nicotine when no acid or alkali was administered immediately before. Sixteen minutes later, sodium carbonate 1.34 per cent, was given. The injection of the same dose of nicotine as before, was followed by a rise of blood pressore from 150 to 176 mm. Hg or 17.3 per cent. After an interval of 25 minutes, the administration of nicotine was repeated, 30 cc. 1 per cent acid having been injected shortly before. Blood pressure increased from 130 to 230 mm. Hg or 76.9 per cent. Several injections made subsequently gave similar results, though the difference between the effect of acid and carbonate on nicotine was less pronounced than after the previous injections. This was also observed in other experiments and will be discussed later. Attention may also be called in this connection to the character of the blood pressure curve obtained. When nicotine was given after acid, the rise was rapid, the ascending limb of the curve being almost vertical. The elevation of blood pressure produced by nicotine after carbonate, was gradual, the ascending limb of the curve making an angle of about 45 degrees with the base line. The recovery was also gradual and was much slower than after nicotine and acid.

Experiment 548, dog, male, 8 kilos. Ether-urethane anesthesia. Two cubic centimeters of a solution of 1–10,000 nicotine injected intravenously (no acid or alkali being given before), raised the blood pressure from 142 to 166 mm. Hg, or about 20 per cent. After an interval of 32 minutes the injection was repeated. The rise of blood pressure was the same as after the first dose, the vagus response was moderately increased, and respiration was stimulated. Twenty minutes later 27 cc. 4 per cent hydrochloric acid were injected, which were followed within 1 minute by 2 cc. of nicotine. Blood pressure was increased 24 per cent, but the effect on the vagus reaction was much greater than after the two previous injections. Approximately the same interval was allowed to elapse and another dose of nicotine was injected after 50 cc. 3.34 per cent carbonate. A sharp fall of blood pressure with instantaneous recovery occurred. The same results were obtained later when the tests were repeated except that cardiac inhibition, after nicotine and acid, was more pronounced than after previous injections.

EXPERIMENTS ON CATS AFTER DOUBLE ADRENALECTOMY. As the observations of some investigators would seem to indicate that nicotine stimulates the secretion of epinephrin, the effect of acid and carbonate on the pressor action produced by it may be attributed to adrenalin. But, according to the experiments of Collip, acid decreases and alkali augments the effect of small doses of epinephrin in dogs. We repeated these experiments on cats and found that the pressor action of adrenalin was substantially the same after acid as after alkali. Indeed, in one experiment it was, on the contrary, increased by acid. This would seem to dispose of adrenal activity as the cause of the different action of nicotine observed when the hydrogen ion concentration of the blood was altered. But the artificial introduction of epinephrin in the circulation may not be the same as increased activity of the adrenals. Experiments with nicotine were therefore made on cats after removing both glands. The results are shown in the following experiment:

A cat weighing 3.4 kilos received, as in previous experiments, urethane and a small amount of ether. The injection of 2 cc. 1–10,000 nicotine half a minute after the administration of 15 cc. 2 per cent hydrochloric acid, was promptly followed by a large vagus pulse lasting 1 minute, and by a fall and then a rise of blood pressure. The injection of the same dose of nicotine 27 minutes later and half a minute after 15 cc. 1.3 per cent sodium carbonate, failed to cause cardiac inhibition or to raise the blood pressure. The injection of nicotine after acid was repeated 30 minutes later, but the pressor action and the response of the vagus were almost the same as after carbonate. Injections of nicotine after acid and carbonate were also made after division of the vagi and the administration of a small but effective dose of atropine. The results were essentially the same as were observed in animals with adrenals intact, that is, there was a considerable rise of bood pressure produced by nicotine after acid and a much smaller rise or no effect when the same amount of nicotine was given after sodium carbonate.

It is evident, therefore, from these experiments, that no causal relations could be found between adrenal function and changes in the behavior of nicotine produced by differences in the hydrogen ion concentration of the blood.

Observations were also made on the vasomotor effect of acid, alkali and nicotine. In oncometric studies on cats, it was noticed that the kidney volume had undergone considerable shrinkage after acid and nicotine, indeed much more than was the case after nicotine alone, while blood pressure showed a corresponding rise. This was especially marked in one experiment in which the contractions of the kidney volume were greater than after the injection of an effective dose of epinephrin. On the other hand, the volume of the kidney was but slightly decreased, and blood pressure was only moderately increased by nicotine after carbonate. It is interesting to note that in some cases there was, on the contrary, an appreciable expansion in the size of the kidney, and a fall of blood pressure after the

injection of carbonate. Acid alone produced no vasomotor effect as the volume of the kidney usually followed the blood pressure.

DISCUSSION. To explain the behavior of nicotine in the body after the administration of acid and alkali, attention may be directed to the following observations:

It has been shown by Pilcher and Sollmann (9), and later by Haskins and Ranson (10), that nicotine stimulated the vasomotor center in the medulla. Since the same effect is produced by small doses of lactic acid as first reported by Mathison (11), and corroborated later by Pilcher and Sollmann (12), the increased pressor action of nicotine after acid may be attributed to the combined effect of these substances. But the volume changes of the kidney, observed after the intravenous injection of acid, failed to support this explanation, for the amounts given produced very little constrictor action, and, in a number of instances, the volume of the kidney closely followed the blood pressure, indicating that the changes were cardiac in origin. The following studies on the heart, however, furnished a more satisfactory explanation.

According to Beyer (13), nicotine is a powerful stimulant of the cat's heart. Haddon and Arrous (14) have shown that the effect is the same on the isolated heart of the dog. Corroborative evidence of the stimulating effect of nicotine on the heart was produced by Fleig (15) and later by Clerc and Pezzi (16) in experiments on the hearts of different animals. Our own observations are in harmony with those cited, for we frequently noticed that small amounts of nicotine injected intravenously into cats caused an improvement in the circulation which lasted for hours. In view of the results obtained by Henderson and Barringer (17), the above observations are of particular interest. According to these investigators, the output of the heart depends in part on the duration and extent of cardiac relaxation. As the capacity of the heart is increased in diastole, a larger volume of blood is accommodated after the injection of acid. The systolic output, when nicotine is injected, is, therefore, increased and blood pressure rises to a higher level. Hence it is the heart rather than the vasomotor mechanism which is the determining factor in the mechanism of the action of nicotine after acid. That the heart also played an important rôle in the behavior of nicotine when given after carbonate, was indicated by the following observations.

Considerable evidence has been obtained by different investigators on the isolated heart showing that increased alkalinity of the perfusate stimulated cardiac tonus. Hence, sodium carbonate, when injected into the circulation, may produce the same effect. The output would thus be decreased and hence the rise of blood pressure would be much less than after acid. That vasomotor stimulation was also less was shown by the fact that the volume of the kidney was only slightly diminished and was

sometimes, on the contrary, increased. It has been observed by Jacobi (18) that hydroxyl ions caused vascular dilatation. The diminished pressor action of nicotine after carbonate was probably caused, therefore, by a decreased output of the heart as well as decreased vascular tonus.

The explanation of the action of acid and alkali on the behavior of nicotine in cats, does not hold, however, when the dose of the latter has been repeated many times. After the total amount injected becomes large, the effect of the nicotine, as shown in experiment 427, was no longer pronounced or was no longer modified by the intravenous administration of acid and alkali, which may be accounted for by the observation of Clerc and Pezzi that prolonged exposure of the heart to the action of the alkaloid diminishes its stimulating effect. The failure of acid and alkali to exert any influence on the action of nicotine in vagotomized dogs, cannot be accounted for in the same way as in cats, since even the first dose of the alkaloid was not affected by a change in the reaction of the blood. Another mechanism, therefore, suggests itself. It will be recalled that respiration in dogs that received urethane was very slow after division of both vagi, which is conducive to accumulation of carbon-dioxide, and probably also to the formation of lactic acid. According to Starling (19), carbon dioxide is very injurious to the dog's heart, while Pilcher and Sollmann (20) have shown that lactic acid causes cardiac irregularity, and a fall of blood pressure. The combined effect of both acids on the heart probably inhibited its reaction to nicotine. The inhibitory effect produced by nicotine, when administered after acid and alkali, may be caused by changes in the medullary center, or in the intracardiac ganglia. But since the reaction of the vagi to faradic stimulation was not influenced by nicotine, nor by acid or alkali, it is obvious that the medullary center was the seat of action of nicotine. The difference in the effect of the alkaloid produced by acid and alkali, may be likewise attributed therefore to changes in the cardio-inhibitory center.

SUMMARY

1. Cardiac inhibition in cats and dogs was increased by small doses of nicotine when injected intravenously after acid, and was decreased or suppressed when similarly administered after alkali.

2. The pressor action of nicotine was greatly increased by the previous administration of acid. It was considerably above that produced by nicotine alone, and was still greater than after nicotine preceded by carbonate. The increased effect was more constant and the difference in the rise of blood pressure was greater after double vagotomy in cats. It was sometimes also observed in dogs.

3. The augmentation of pressor effect was attributed to the greater output of the heart caused by lower tonus due to increased hydrogen ion concentration and to the stronger contractions produced by nicotine.

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3. The augmentation of pressor effect was attributed to the greater output of the heart caused by lower tonus due to increased hydrogen ion concentration and to the stronger contractions produced by nicotine.

4. Vasomotor action as a factor in determining the effect of nicotine in these conditions was discussed.

5. The decrease or absence of pressor action observed when nicotine was injected after carbonate, was explained by the decreased output of the heart due to stimulation of cardiac tonus and to dilatation of the peripheral vessels caused by hydroxyl ions.

6. Adrenalectomy failed to exert any influence on the effect of acid and alkali on nicotine.

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A STUDY OF HEAT PRODUCTION IN PIGEONS ON DIETS DEFICIENT IN VITAMIN B[1]

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Eijkman (1897) in his paper on polyneuritis in fowl, makes the interesting observation that "the body temperature drops a few degrees below normal." At the time the investigation reported here was planned (1918), no calorimetric observations had been made as to the exact decrease in heat production of an animal on a diet deficient in vitamin B. Experiments were therefore started in which the heat production of rats on diets deficient in vitamin B were measured by means of a calorimeter of special construction. For reasons which are stated later, this procedure was abandoned and the investigation commenced once more using pigeons and a calorimeter of standard design. Accordingly, pigeons of known age were placed on one of the three diets, deficient in vitamin B, described below. Calorimetric measurements were made during the period of depletion of body vitamin; at the onset of the convulsive attack of polyneuritis columbarum; and finally after the administration of a curative dose of yeast concentrate either alone or with food. In some cases the observations were continued until a normal heat production and body temperature had been reëstablished, as indicated by a comparison with control birds.

During the last five years a number of papers have been published concerning heat production in polyneuritis.

Novaro (1920a, b, c) reported observations upon the decrease in body temperature during avian polyneuritis, together with data relative to loss in body weight and heat production as measured by means of a D'Arsonval compensating calorimeter.

Abderhalden (1920) prepared extracts from yeast using alcohol and acetone. The effect of the material so obtained was noted upon the body weight, temperature and length of life of pigeons maintained exclusively upon polished rice.

[1] The data upon which this paper is constructed form a part of a thesis presented to the Board of Graduate Studies, Northwestern University, by Herman Edward Redenbaugh in May, 1924, in partial fulfilment of the requirements for the degree, Doctor of Philosophy.

Abderhalden and Schmidt (1920), following the observation of Myerhof (1918a, b) that saline extracts of yeast increased muscular respiration in normal animals, prepared extracts of yeast both saline and by means of alcohol and acetone and observed the influence of these upon the oxygen consumption by breast muscle of normal and polyneuritic pigeons. They observed a marked decrease in the oxygen consumption by muscle from polyneuritic birds as compared with that from normal pigeons. The action of yeast extract was variable. It did not increase the oxygen consumption of normal muscle to any great extent, while the application to muscle from polyneuritic pigeons showed a marked increase in one or two cases. When a polyneuritic pigeon was anesthetized (ether) and one leg removed, a potent yeast extract then injected, complete recovery of the pigeon resulting, and the other leg subsequently removed, they report no demonstrable difference in oxygen consumption by muscle of the two legs respectively.

Abderhalden (1921a) measured also the carbon dioxide production by pigeons using Weil's modification of the Haldane method. His results show a progressive decrease in carbon dioxide production (grams CO_2 per kilogram per hour) during the period of feeding pigeons on an exclusive diet of polished rice. When yeast pills, made from one of his yeast extracts and cooked rice, were given to the polyneuritic pigeons, an increase in carbon dioxide production occurred within one to two hours.

McCarrison (1921) in his extended study of pigeons and monkeys with polyneuritis reports the characteristic decrease in body weight and temperature mentioned by other investigators, and in addition gives a most complete account of the histological changes occurring in various organs. Previous to this, changes in the gastro-intestinal tract had been mentioned by Eijkman and by Voegtlein and Lake (1918), the latter authors working with cats and with dogs.

Calorimetric investigations of fowl fed on exclusive diets of polished rice were made by Anderson and Kulp (1922). They state that the respiratory quotient during vitamin B starvation does not vary much from normal, thereby concurring with Jansen and Mangkoewinoto (1920) and differing from Ronoino (1914). The heat production, however, decreased in some cases as much as 50 per cent below the basal level, due in part undoubtedly to a lowered food consumption. Data are also given for heat production during the convulsive attack of polyneuritis and in the case of one hen during the period of recovery.

Findlay (1923) notes a greater susceptibility to infection in polyneuritic animals, this running parallel to the temperature drop. McCarrison (pp. 44–45) had previously pointed out the importance of this factor, stating that it may "operate in one of two ways: it may precipitate the onset of symptoms due to food deficiency, or it may impart new clinical features to the food deficiency syndrome."

Finally Gulick (1924) reports a decreased energy output when rats are placed on a diet of starch, casein, butter and salts. His controls on a similar diet with the addition of 0.2 gram yeast showed, according to the percentage of adult normal metabolism, almost as great a decrease. He mentions the fact that both sets were under-nourished. We cannot see that he distinguishes between the effects of under-nourishment and of polyneuritis on the decrease in basal metabolism.

Specific reference to some of the above literature will be made in discussing our results.

Calorimetry. As mentioned above, it had been our intention to use white rats in this investigation. As the gaseous metabolism of these animals is small (about 4 cc. oxygen per minute), a calorimeter was constructed of glass parts, using large test-tubes for the absorbing system and a 50 cc. graduated burette with a 250 cc. bulb blown below the calibations to measure the oxygen consumption. A small glass chamber contained the animal, while the circulation of air was maintained by means of a Cenco blower. Owing to technical difficulties this plan was abandoned.

We next sought to adapt the standard Universal Calorimeter of F. G. Benedict (1912), provided with a small glass chamber, to our needs, using pigeons for the experimental animal. Although our results were satisfactory, the difficulty of readily securing a perfect seal between chamber and cover led to the adoption of a small copper chamber, 8 inches square (inside measurements) sealed by means of a trough 4 inches deep which was filled with water. The chamber was similar in all other respects to that recommended by Benedict (1915) for respiration work with small animals.

The ventilation rate of the chamber was regulated to between 1 and $1\frac{1}{2}$ liters per minute, by means of a by-pass valve connected between the exhaust and intake sides of the Crowell blower. Oxygen was measured in a detached spirometer of 9 liters capacity, and fed into the circuit through a Benedict oxygen trap. The apparatus was repeatedly tested for leaks and for completeness of absorption of carbon dioxide and water vapor in the usual manner.

At frequent intervals during the course of the investigation "ether checks" (Carpenter, 1915) were run with the burner connected to the circuit in place of the animal chamber. The following data, table 1, are recorded for the sake of completeness. The lowest check obtained was 0.6559.

Before starting an observation, the pigeon was carefully weighed. The wings and feet were secured to the body by wrapping loosely with a few turns of wide bandage. The temperature taken per cloaca was recorded, and the animal placed in the chamber. The ventilating circuit was then started, the unweighed set of absorbers being used. After

15 to 20 minutes the barometer was read, the gas temperature was taken, both in the circuit and the spirometer, and finally the pressure in the circuit recorded as shown on a water manometer. If the above measurements remained constant for a period of 10 minutes, the observation period was commenced, using the duplicate set of absorbers which had been previously weighed on a large balance to an accuracy of 10 mgm.

TABLE 1

Ether checks

Duration of period—15 minutes

	FOUND	THEORY
Test 3:		
Cubic centimeters carbon dioxide	1,894.0	
Cubic centimeters oxygen	2,856.0	
Respiratory quotient	0.664	0.666
Test 8:		
Cubic centimeters carbon dioxide	2,240.0	
Cubic centimeters oxygen	3,376.6	
Respiratory quotient	0.663	0.666

Experimental work. Pigeons are well suited to an investigation of this character because of the rapidity with which they develop polyneuritis, and the striking manner in which the symptoms are manifest. They consume relatively large amounts of oxygen per unit of body weight. According to Sugiura and Benedict (1923), they do not require vitamins A or C. They do not eat flies or other vermin which might serve as a source of vitamin. They remain quiet in the dark calorimeter chamber.

Our pigeons were of the common type, being purchased on the open market. When received they were approximately three weeks old, the voice changing about one week later. Their bodies were about half feathered out. The pigeons were placed in metal cages of ample size, usually two birds to a cage. The cages were kept in a large, well-ventilated room, and each day were cleaned and supplied with water, quartz sand and coarse ground charcoal.

Diets. The pigeons were placed for two weeks on a commercial chicken feed diet consisting of corn, Kaffir corn, wheat, oats, buckwheat, barley, hemp and sunflower seed. At the end of this period the birds were divided into six groups and placed on their respective diets.

Three diets were prepared, the first two were the synthetic lard and the synthetic butter diets of Sugiura and Benedict (1923) (see table 2) while the third was the previously mentioned grain diet autoclaved for 8 hours at 20 pounds pressure.

The salt mixture used was that designated by Osborne and Mendel (1913) as number 4 without lactose. Yeast vitamin B was supplied to the controls in the quantity indicated in the brackets.

The casein was purified in the following manner. Commercial casein was dissolved in a large volume of water by means of ammonia. It was then precipitated by dilute acetic acid, and washed four times with distilled water by decantation. This process was repeated three times. The major part of the water was removed by filtration on a Buchner funnel. The material was washed with alcohol, and finally sucked dry in the funnels. The casein was then placed in a 12-liter Pyrex flask and the remaining fat removed by extraction with hot alcohol in a continuous extractor for one week.

The cornstarch was subjected to a similar alcohol extraction, while the agar-agar and cane sugar were autoclaved in tight containers for eight hours. The butter was purified by melting at 45°C. and filtering through paper in a hot water funnel. The filtrate was then centrifuged at high speed for 1 hour, after which the clear oil was decanted off. The lard was purified by first melting, and then pouring into a large volume of

TABLE 2

Basal diets (Sugiura and Benedict)

	SYNTHETIC BUTTER DIET—VITAMIN A ONLY	SYNTHETIC LARD DIET—NO VITAMIN
	grams	*grams*
Casein	22	22
Cane sugar	10	10
Cornstarch	27	37
Agar-agar	2	2
Salt mixture	3	3
Butter fat	30	0
Yeast	(6)	(6)
Lard	0	20

absolute alcohol at 60°C. It was allowed to cool over night, and then filtered. This procedure was repeated three times, and the remaining alcohol finally being removed by evaporation on the steam bath.

The yeast used as a source of vitamin B was prepared by the Harris Laboratories, and sold commercially as "Dried Powdered Yeast Tested." This was added to the diet of the controls. The "Concentrated B vitamin, from Yeast, Bacteriological" prepared by the same Company, was used for curative purposes.

Fifteen grams of the synthetic butter diet, 20 grams of the synthetic lard diet, and 25 grams of grain were given to each pigeon of the respective groups per day. Forced feeding was necessary in all cases except with the controls on a grain diet. The feeding was accomplished by placing the pigeon in a stanchion made from a box slightly larger than a chalk box. The synthetic diet was made into a paste with 20 cc. of distilled

water, and placed in the pigeon's crop by means of a large metal syringe, using a small catheter as a stomach tube. To get the autoclaved grain into a condition so that it would pass through the stomach tube, it was necessary to grind in a mill and boil with water for a few minutes.

Calorimetric determinations. Each observation period was 3 hours in length. The pigeons had received no food for the previous 18 to 22 hours. As a rule, an animal under observation was fed just after being removed for the day from the calorimeter chamber. As an investigation of the nitrogenous metabolism during the calorimetric period was not made, the heat production was calculated using the caloric value of oxygen corresponding to the total respiratory quotient of that period as given in the tables of Zuntz and Schumburg and modified by Lusk (1917). We realize that an error is introduced by this procedure, but in pigeons with polyneuritis food is retained in the crop and probably also in the elemen-

TABLE 3

Onset of polyneuritis columbarum

(Number of days on experimental diet before convulsive attack)

	DIET						
	Autoclaved grain		Synthetic butter			Synthetic lard	
Pigeon number	12	15	7	8	11	5	6
	days	*days*	*days*	*days*	*days*	*days*	*days*
First attack	31	23	26	19	21	14	17
Second attack				10	12	10	9
Third attack				7		11	

tary tract for long periods of time. It is doubtful that failure to consider nitrogenous metabolism, or to make the usual allowance of 15 per cent of calories from protein in the post-absorptive period, would change the significance of our results, since there is reason to ǝʌǝi̯ǝq that the food in the alimentary tract may under these conditions exert an unknown and highly variable specific dynamic factor. Our figures for heat production must therefore be taken as relative values.

The calculations of the respiratory quotient and caloric output were made in the usual manner, but omitting the protein factor.

Tabulation of results and discussion. The pigeons were used in this investigation as follows:

Starvation ..... nos. 1–2
Controls:
Mixed grain ..... nos. 17–18
Butter diet plus yeast ..... nos. 4–14–19
Lard diet plus yeast ..... nos. 9–10

Vitamin deficient diet:
Autoclaved grain..... nos. 12–15
Butter diet, no yeast..... nos. 7–8–11
Lard diet, no yeast..... nos. 5–6

The time of onset of polyneuritis columbarum. As the pigeons were all of about the same age, and as they were kept on the same mixed grain diet previous to starting the experimental diets, it seems reasonable to assume that the vitamin content of their bodies must be about equal, relative to a unit of body weight. If this assumption is correct, any difference in the time of onset of the convulsive attack among the polyneuritic pigeons on different experimental diets would be of significance, provided that the food intake was approximately the same per unit of weight.

Table 3 shows the difference in time of onset of polyneuritis, the exhaustion of body vitamin occurring in the groups in the following order, —first, the synthetic lard diet; second, the synthetic butter diet; third, the autoclaved grain diet. Although the pigeons on the lard diet received 5 grams more food per day than those on the butter diet, this fact can hardly be responsible for the more rapid onset of polyneuritis. Pigeons of this group regurgitated more of their food and had a more severe diarrhea than did any of the others. The loss in weight of pigeons 5 and 6 was 7 and 5 grams respectively, their initial body weight differing but 20 grams.

The pigeons on the synthetic butter diet required a longer period for the exhaustion of body vitamin. That a difference in body weight has no great influence upon the time of onset is shown by pigeons 8 and 11, in the following tabulation:

	PIGEON 8	PIGEON 11
Initial weight	330 grams	450 grams
Weight at onset	306 grams	410 grams
Loss in weight	24 grams	40 grams
Time on experimental diet	19 days	21 days

Each of the above pigeons received the same quantity of food (15 grams per day). Although the difference in initial body weight was 120 grams, yet the period of onset agreed within 2 days. The smaller amount of food per unit of body weight received by pigeon 11 was without significance except as possibly shown in a greater loss of body weight. It certainly did not delay the onset of the attack.

It is noteworthy that the pigeons on the autoclaved grain diet exhausted their body vitamin more slowly than did either of the other groups. Although receiving the most food (25 grams) and frequently regurgitating a considerable amount, the nature of the diet was, however, such as to

furnish a roughage which was lacking in the two concentrated diets. The delayed onset of polyneuritis in this group may have been due to this factor and not to the presence of vitamin in the grain. Again, the difference may have been due to the character of the diets. The time required by this group for exhaustion of body vitamin agreed well with that observed by other investigators, when feeding polished rice (Abderhalden 21 to 33 days; McCarrison 17 to 33 days).

After measuring the respiratory quotient and heat production at the convulsive attack of polyneuritis, the pigeon was immediately given a curative dose of 0.5 gram of "Concentrated B Vitamin from Yeast," Harris. After an interval of about an hour, the pigeon was returned to the colorimeter and the observations continued. The following day the pigeon was placed on its previous experimental diet. Table 4 presents data showing the exhaustion of this curative dose of vitamin B. It is

TABLE 4

Temperature of control pigeons on adequate diets

(Fahrenheit degrees)

	GRAIN DIET		SYNTHETIC BUTTER AND YEAST			SYNTHETIC LARD AND YEAST	
Pigeon number	17	18	4	14	19	9	10
Initial temperature	107.8	107.6	107.0	107.8	107.0	108.4	107.8
Final temperature	108.0	107.4	107.5	107.8	108.2	107.0	107.6
Group average	107.7		107.8			107.7	

interesting to note that the periods of exhaustion this time are shorter in both groups, and also that the periods of onset are in closer agreement. Data are presented for a third period of exhaustion of one pigeon on each synthetic foodstuff. This period followed the administration of a second curative dose of 0.5 gram of yeast concentrate, and the resumption of the experimental diet.

Influence on body temperature. The normal cloacal temperature of our pigeons was 107.7°F. (McCarrison—average of 129 observations gave 107.4°F.). That it does not vary from this point under conditions of widely different dietaries, and even when forced feeding is used, providing that the diet is adequate in respect to vitamin requirement, is shown in table 4. The initial temperatures recorded in this table are those of the pigeons on the first or second day of the diet, while the final temperatures were those on the last day of the investigation.

In contrast to the uniform temperatures of the controls, table 5 gives the temperature variations of the polyneuritic pigeons on diets similar in all respects to those of the controls, but lacking vitamin B.

A remarkable temperature drop of 16.0 was recorded for pigeon 15. This individual drop gives a greater group decrease to the two pigeons on the autoclaved grain diet than is exhibited in either of the other two groups. Excluding this exceptional case, it is seen that the average decrease in temperature at the onset of polyneuritis is about 6°F. As pointed

TABLE 5

Temperatures of pigeons on diets deficient in vitamin B

(Fahrenheit degrees)

	AUTOCLAVED GRAIN DIET		SYNTHETIC BUTTER, NO YEAST			SYNTHETIC LARD, NO YEAST	
Pigeon number	12	15	7	8	11	5	6
Temperature at start of deficient diet	107.0	108.0	107.8	107.0	107.6	108.0	107.8
Temperature at onset of convulsions	102.8	92.0	99.0 (died)	100.0	103.2	101.6	102.0
Drop in temperature, group average	10.1		6.7			6.1	
Temperature 18 hours after 0.5 gram yeast concentrate and food				106.4	106.4		106.6
Temperature 18 hours after 0.5 gram yeast concentrate only		104.0*				104.6	

* Temperature was 104.0 six hours after giving 0.5 gram yeast concentrate. It, however, did not return to normal under the influence of vitamin alone.

TABLE 6

Temperature during starvation

	PIGEON 1	PIGEON 2
	°F.	°F.
Initial temperature	107.8	107.8
Temperature after 11 days' fast	101.6	101.8
Drop in temperature	6.2	6.0
Temperature 2 hours after 15 grams butter diet and yeast	104.8	104.6
Temperature 18 hours after above meal	105.0	105.0
Temperature 48 hours later, fed 2 meals	108.4	106.4

out by most observers, the body temperature shows no decrease during the first 7 to 10 days on a vitamin B free diet. Pigeon 5 on the lard diet was an exception, showing a pronounced decrease in 4 days during each of the three depletion periods.

It is interesting to observe the rapidity of increase in body temperature

under the administration of food and a curative dose of yeast concentrate; and also after giving 0.5 gram of yeast concentrate alone. With food and yeast, the body temperature returned nearly to normal (106.4°F. average) in 18 hours, while with vitamin alone, it had increased considerably, returning to normal only after the pigeon had been returned to a natural grain diet.

Pigeons on a diet deficient in vitamin B retain increasingly larger amounts of food in their crops, until at the onset of the acute symptoms of polyneuritis, the crop usually is greatly distended. They appear to be starving, yet engorged with foodstuff. The similarity to starvation, respective to body temperature, is shown in table 6. McCarrison has reported a similar drop in body temperature, culminating in death usually in 12 days, with a temperature around 99° to 96°F. No evidence of polyneuritis was present.

By a comparison of tables 5 and 6 it is apparent that pigeons experience during 11 days of starvation a drop in body temperature that is almost exactly the average drop for polyneuritic pigeons at the onset of convulsions. Furthermore, the response as manifest in a rise in temperature when the starved pigeon is given food and vitamin is extremely rapid, reaching in 2 hours a point almost as high as that attained 18 hours after the administration of 0.5 gram of yeast concentrate to the polyneuritic pigeon.

In comparing these two groups, it is to be remembered that the gastrointestinal tract of the starved pigeon is normal and empty with little or no permanently decreased efficiency of the digestive enzymes. In the pigeons with polyneuritis, the crop is full of food and the gut usually shows degenerative changes. It is therefore not surprising that starved pigeons respond rapidly to the stimulus of food. The increase in body temperature noted when the polyneuritic pigeon is given 0.5 gram of yeast concentrate must either enable digestion of retained food to commence, or else increase tissue oxidation. We will present evidence in the next paper to show that the digestive enzymes of a polyneuritic pigeon have suffered a great decrease in efficiency. As pointed out in the first part of this paper, Abderhalden observed a marked decrease in the oxygen consumption of breast muscle from polyneuritic pigeons as compared with similar tissue from normal birds. Dutcher (1918) has observed a great decrease in the catalase content of the tissue of polyneuritic birds, and ascribes the reduction in oxidation to a decreased supply of vitamin B.

Influence upon body weight. Table 7 shows the gain in weight in the control pigeons on their respective diets.

Considerable individual variation is observed in the increase in weight of the control pigeons. The group average gains are about the same for the butter and lard diets, and slightly higher on mixed grain.

In table 8 the loss in body weight of the pigeons on the three diets free from vitamin B are recorded.

A striking difference is apparent between group average losses on the three types of foodstuffs. The groups in order of increasing loss in body weight are synthetic lard, synthetic butter, and autoclaved grain. It is important to note that this is the order of onset of the convulsive attack of polyneuritis as indicated in table 3.

TABLE 7

Increase in body weight of control pigeons

	DIETS						
	Grain		Synthetic butter with yeast			Synthetic lard with yeast	
Pigeon number	17	18	4	14	19	9	10
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Weight at start	328	292	342	290	415	330	355
Final weight	400	470	390	450	490	465	420
Increase	72	178	48	160	75	135	65
Group average increase	125		94.3			100	

TABLE 8

Decrease in body weight of polyneuritic pigeons

	DIETS						
	Autoclaved grain		Synthetic butter, no yeast			Synthetic lard, no yeast	
Pigeon number	12	15	7	8	11	5	6
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Initial weight	327	370	351	330	450	360	380
Weight at first acute attack	220	275	340	306	390	353	350
Loss in weight	107	95	11	24	60	7	30
Group average loss	101		31.7			18.5	
Group average, daily loss	3.7		1.54			1.15	

The loss in body weight during starvation is shown in table 9.

McCarrison reported a daily loss in weight during starvation of 8.3 grams for male pigeons and 10.4 grams for females. His values are the averages of a considerable number of observations. By a comparison of tables 8 and 9, it is seen that the average daily loss in body weight during starvation is much greater than that occurring during the depletion period while the pigeons are on diets free from vitamin B.

Influence on respiratory quotient. The calorimetric observations were

started shortly after 9 o'clock in the morning, and usually extended until early afternoon. The pigeons were then fed for the day. By this procedure, the birds were in as near a post-absorptive state while in the calorim-

TABLE 9

Decrease in body weight during starvation

(Duration of fast—8 days)

	PIGEON 1 (FEMALE)	PIGEON 2 (MALE)
	grams	*grams*
Initial weight	290	320
Final weight	190	240
Loss in weight	100	80
Average daily loss	9.1	7.3

TABLE 10

Respiratory quotients—Control pigeons on adequate diets

GRAIN DIET		SYNTHETIC BUTTER WITH YEAST			SYNTHETIC LARD WITH YEAST	
Pigeon 17	Pigeon 18	Pigeon 4	Pigeon 14	Pigeon 19	Pigeon 9	Pigeon 10
0.83	0.84	0.76	0.88	0.98	0.85	0.88
0.82	0.82	0.88	0.91	0.99	0.80	0.82
0.83	0.81	0.94	0.90	0.90	0.81	0.82
				0.91		0.80

TABLE 11

Influence of vitamin B upon the respiratory quotient

	DIETS						
	Autoclaved grain		Synthetic butter, no yeast			Synthetic lard, no yeast	
Pigeon number	12	15	7	8	11	5	6
During body depletion	0.84	0.82*	0.85	0.72			
	0.63	0.69	0.78	0.73			
			0.77	0.73	0.98	0.80	0.95
At onset of convulsions	0.60	0.69	0.75	0.69	0.74	0.69	0.71
After 0.5 gram yeast concentrate	Died	0.90	Died	0.75	0.97†	0.73	0.97
After 24 hours (1 feeding)		0.74‡		0.97	1.03	1.03	1.01

* On a natural grain diet.

† Received 0.5 gram yeast concentrate and 15 grams butter diet 5 hours previous.

‡ Received 0.5 gram yeast concentrate only.

eter as possible under the conditions existing in polyneuritis columbarum. The only exception to the above procedure was in the case of a pigeon receiving a curative dose of yeast concentrate, with or without food, and

being returned to the calorimeter in about two hours for further observation.

The respiratory quotients of the control pigeons on adequate dietaries are given in table 10. They are recorded here in the order in which they were determined.

The pigeons on the grain diet were allowed to eat whatever quantity of food they desired, while those on the butter and on the lard diets were fed definite amounts once a day by means of a syringe and stomach tube. This undoubtedly accounts for group differences. In general, the respiratory quotients remained at about the same level for the respective pigeon throughout the investigation.

The influence of vitamin B upon the respiratory quotient is clearly brought out in table 11.

The above table shows an unmistakable lowering of the respiratory quotient, with the depletion of body vitamin. We therefore cannot agree with Jansen and Mangkoewinoto (1920) or with Anderson and Kulp (1922) that the respiratory quotients during vitamin starvation, and at the onset of the convulsions, do not vary much from the normal. Our results however substantiate the findings of Romoino (1914).

The effect of a curative dose of yeast concentrate in raising the respiratory quotient is very pronounced. After an interval of 24 hours, the body regains to a remarkable extent the ability to oxidize carbohydrate as indicated by a respiratory quotient of approximately 1. This has an interesting bearing in connection with the observed decrease in efficiency of carbohydrate splitting enzymes of the gastro-intestinal tract of polyneuritic pigeons as will be brought out in the following paper. It also raises the question as to the specific site of influence of vitamin B. Does it act in conjunction with the digestive enzymes, or with the specific tissue ferments? From the evidence presented by Dutcher (1918) and by Abderhalden (1920, 1921 a, b), together with that stated here and in the following paper, it is our belief that vitamin B has a marked influence upon enzymes both of the digestive tract and those effective in tissue oxidation.

The most remarkable depression of the respiratory quotient occurred during starvation as is shown in table 12.

In the case of the above pigeons, no evidence of polyneuritis was observed. A marked acidosis must have occurred, for when pushed over, the pigeon gasped for breath while regaining its feet. As has been pointed out by Magnus Levy (Lusk, 1917, p. 471) a possible reduction of the respiratory quotient may occur when beta-oxybutyric acid is formed from fat. Here certainly the tissues are lacking all stimulation from food nutrients, a condition which may not be as absolute in polyneuritis. That no permanent injury to the digestive or tissue enzymes occurs during

TABLE 12

Respiratory quotients during starvation

	PIGEON 1	PIGEON 2
While on grain diet	0.84	0.83
Starvation	0.79	0.84
	0.66	
Eleventh day of fast*	0.54	0.57
Eleventh day—2 hours after feeding †	0.68	0.93
24 hours after feeding	0.94	0.97

* Pigeons weak and unable to stand.

† Calorimetric period 2nd to 5th hour after feeding 10 grams of butter diet, the same as fed to controls.

TABLE 13

Heat production—control pigeons

Calories per square meter per 24 hours

DIETS						
Grain diet		Synthetic butter with yeast			Synthetic lard with yeast	
Pigeon 17	Pigeon 18	Pigeon 4	Pigeon 14	Pigeon 19	Pigeon 9	Pigeon 10
880	860	840	950	728	960	996
880	880	880	940	712	(1,020)	1,020
863	880	907	980	1,040	960	960
				880		1,040
				880		

TABLE 14

Influence of vitamin B upon heat production

Calories per square meter per 24 hours

	DIETS						
	Autoclaved grain		Synthetic butter, no yeast			Synthetic lard, no yeast	
Pigeon number	12	15	7	8	11	5	6
During body depletion	780	840*	880	960	820	840	770
		520	790	700			
			630	730			
At onset of convulsions	464	610	660	690	690	710†	610
After 0.5 gram yeast concentrate	Died	740	Died	810	780	770	790
After 24 hours		610		830	800	1,030	760‡

* On whole grain.

† Convulsions started about one half hour after completion of this determination.

‡ Pigeon continued on vitamin free diet.

starvation is indicated by the response obtained upon administration of food. This response is additional proof that the body during starvation conserves its supply of vitamin (even vitamin B) for the quantity of dried yeast contained in the butter diet given is far lower in vitamin content than that obtained by a polyneuritic pigeon receiving a curative dose of 0.5 gram of yeast concentrate.

TABLE 15

*Hen 4,—Anderson and Kulp**

	WEIGHT	O_2 PER HOUR	R.Q.	ESTIMATED CALORIES PER HOUR	ESTIMATED CALORIES PER SQUARE METER PER 24 HOURS	CONDITION
			Normal basal metabolism			
	grams	*grams*				
6/ 8/21	1,700	1.344	0.87	4.59	744	Moved
			Metabolism during vitamin starvation			
7/26/21	1,410	0.808	0.76	2.68	492	Quiet
8/ 1/21	1,350	0.877	0.76	2.92	552	Restless
			Metabolism during polyneuritis			
8/ 3/21	1,305	0.978	0.75	3.24	626	Convulsion
			Metabolism after recovery from polyneuritis			
8/11/21	1,325	1.504	0.76	4.99	955	Restless
10/ 2/21	1,465	1.288	1.03	4.51	807	Quiet

* Anderson, R. J. and W. L. Kulp, 1922, p. 87.

TABLE 16

Heat production during starvation

Calories per square meter per 24 hours

	PIGEON 1	PIGEON 2
While on grain diet	860	880
	620	640
Starvation	410	
11th day of fast	400	460
11th day (after feeding)	510	530
24 hours after feeding	1,040	880

The effect of vitamin B deficiency upon heat production. The heat productions of the control pigeons on the three adequate diets are given in the sequence in which they were determined in table 13.

The average value of all controls is 915.7 calories per square meter per 24 hours. E. Voit (Lusk, 1917, p. 41) obtained 947 calories per square meter per 24 hours for a fowl of 2 kilograms weight.

The influence of vitamin B upon heat production is shown in table 14, the values being tabulated in sequence of determination.

The decrease in heat production during the period of depletion of body vitamin is apparent from the above figures. Considerable individual variation exists, but in general the picture is similar in all cases. At the onset of convulsions in the polyneuritic pigeons, the average of all values for heat production is 633 calories per square meter per 24 hours. The average heat production is therefore but 68 per cent of the normal. The increase in heat production following the administration of 0.5 gram of yeast concentrate is shown to be very definite.

Our results for heat production in pigeons upon a vitamin B free diet are similar to those of Anderson and Kulp for hens on an exclusive diet of polished rice. One of their fowls (no. 4) survived the period of convulsion and was restored to normal. As Anderson and Kulp expressed the heat production of their hens in calories per kilo per hour, we have for comparison calculated some of the values for hen 4 to calories per square meter per 24 hours. It is unfortunate that they did not determine the heat output of this hen 24 hours after giving yeast extract with food. It is probable that they would have found an increased respiratory quotient, as well as a normal heat production. The data are given in table 15. It will be noted that our average results for pigeons are slightly higher than those cited by them in this instance.

It should be mentioned here that Novaro (1920c) expressed the heat production of pigeons upon an exclusive diet of polished rice in terms of calories per unit of surface area. Unfortunately, in the use of the Meeh formula, she neglected to include the value of the constant k, which for fowl is 10.4. Her average value for surface area is about 50 square centimeters, which would be the area of a 2.9 centimeter cube.

The heat production during starvation is shown in table 16.

It is striking that a lower heat production occurs during starvation than in polyneuritis. The response to food is about equal in both cases. It must however be remembered that the polyneuritic pigeon required a curative dose of 0.5 gram of yeast concentrate to effect this response, whereas in the starved pigeon the increase was due to food alone.

Note: The synthetic butter diet fed to the starved pigeons 1 and 2 contained dried powdered yeast in sufficient quantity only to make it adequate for maintenance and growth. This, as mentioned before, is not to be compared with the vitamin content of the 0.5 gram of yeast concentrate received by the polyneuritic pigeons.

SUMMARY

Young pigeons about one month old, were placed on one of the three following diets: autoclaved grain, Sugiura and Benedict's butter diet, and Sugiura and Benedict's lard diet; all of which were lacking in vitamin

B. Controls were maintained on whole grain and the diets of Sugiura and Benedict, with the addition of yeast powder. Another group of pigeons was subjected to starvation, water being allowed.

The time required to deplete the body of its vitamin content on each of the deficient diets was noted, together with changes occurring in body weight, temperature, respiratory quotient and heat production. Comparisons were made with the respective controls as well as with the group undergoing starvation.

The following facts are clearly brought out:

1. The exhaustion of the body supply of vitamin B occurred in the groups in the following order,—first, synthetic lard diet; second, synthetic butter diet; third, autoclaved grain diet. It should be mentioned here that the pigeons on both the autoclaved grain and lard diets received no vitamin, while those on the butter diet received vitamin A. The second period of vitamin depletion, following the administration of a curative dose of yeast concentrate and resumption of the deficient diet, was noticeably shorter in all cases.

2. The decrease in body temperature during vitamin depletion averaged 6°F. When a curative dose of 0.5 gram of yeast concentrate was given, the body temperature rose rapidly showing a marked increase in a few hours. If food was given along with yeast concentrate, the temperature returned nearly to normal in 18 hours. The temperature of a pigeon on the 11th day of starvation was almost exactly the same as a polyneuritic pigeon at the onset of convulsions. The rise in temperature of the former on food alone reached in two hours a point almost as high as was attained in 18 hours by the administration of 0.5 gram of yeast concentrate to the polyneuritic pigeon.

3. The loss in body weight of the polyneuritic pigeons followed the same order as the period of depletion of body vitamin, being least in the lard group and greatest in the autoclaved grain group. The average loss per day during starvation was greater than that of a similar period during the depletion of body vitamin.

4. The respiratory quotients of individual pigeons on adequate diets maintained a marked constancy, yet showed variations among the groups. With pigeons on diets deficient in vitamin B, there is a progressive lowering of the respiratory quotient. Contrary to the findings of Jansen and Mangkoewinoto, and of Anderson and Kulp, our respiratory quotients were lower at the onset of convulsions than at any other time. In this respect we confirm the earlier work of Romoino. Administration of a curative dose of 0.5 gram of yeast concentrate markedly increased the respiratory quotients. After a period of 24 hours the respiratory quotient indicated that the pigeon was able to oxidize carbohydrate. The quotient was much higher than the average for the corresponding con-

trol. It is suggested that vitamin B influences the activity of both digestive and tissue enzymes. The respiratory quotient decreased during starvation to a point lower than that attained by any pigeon on an inadequate diet. The increase in response to food was rapid.

5. The average of all values for heat production of control pigeons on adequate diets was 921 calories per square meter per 24 hours. At the onset of convulsions the polyneuritic pigeons showed an average of but 633 calories. This represents a heat production of but 68.7 per cent of the normal. During starvation the heat production decreased markedly. On the 11th day the average was but 430 calories per square meter per 24 hours. The response to food was rapid, the heat production returning to normal in 24 hours.

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THE DECREASE IN DIGESTIVE EFFICIENCY IN POLYNEURITIS COLUMBARUM[1]

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The most remarkable phase of polyneuritis columbarum is the recovery following the administration of some source of vitamin B. This recovery is unmistakable, for a pigeon which to all appearances is a helpless moribund mass, will in a few hours after the administration of small amounts of yeast concentrate, frequently regain its feet and show an increase in body temperature and heat production. According to Dutcher (1918), the tissue of a polyneuritic pigeon has a much lower catalase content than normal. That a lower consumption of oxygen occurs in polyneuritis, has been shown to be true for breast muscle by Abderhalden and Schmidt (1920). In this paper we purpose to show that the pancreatic enzymes responsible for the digestion of carbohydrate, fat and protein in the intestine, have also suffered a marked decrease in efficiency. This decrease furthermore is observed in the activity of a rennin-like enzyme of the stomach. We conclude by inference that during recovery the influence of vitamin B must at least in part be directed at a restoration to activity of these enzymes, both of digestion and of tissue oxidation.

The fact that the glands and organs of the body atrophy during vitamin B privation is well known. Funk and Douglas (1914) reported that all glands atrophy and degenerate when the organism is placed on a vitamin-free diet. McCarrison (1921) has made an elaborate study of the pathology resulting from polyneuritis in pigeons and monkeys. He notes the marked decrease in weight of the pancreas, and when studied histologically finds that the "texture of the organ is less open, its lobules are less distinct, and its parenchyma-cells appear sunken and collapsed. The alveoli are more closely packed, and the sunken cells appear to be functionally less active; they seldom exhibit granular evidence of an efficient output of work" (p. 128). Besides the general atrophy of the gastro-intestinal

[1] The data upon which this paper is constructed, form a part of a thesis presented to the Board of Graduate Studies, Northwestern University, by Herman Edward Redenbaugh in May, 1924, in partial fulfilment of the requirements for the degree, Doctor of Philosophy.

tract, he gives this picture of the mucous membrane of the upper part of the intestine,—"(a) atrophy and partial or complete disappearance of the villi; (b) frequent congestion of such villi as remain; (c) atrophy of the glandular cells of the crypts of Lieberkuhn, and separation of these from their basement membrane; etc." (p. 91). This will suffice to emphasize the extent of the structural changes in the severe cases of polyneuritis.

The dependence of many glands and organs upon vitamin B for their normal function, has been observed by the following investigators. Uhlman (1918) states that vitamin B stimulates glands, acting through the nervous system similar to pilocarpine. Dutcher (1920) believes that the ductless glands are stimulated by vitamins in an unknown manner, and that the body may be dependent upon them to provide it with hormones. Previous to this, Voegtlin and Myers (1919) sought to prove the identity of vitamin B and secretin. As pointed out subsequently by Cowgill and Mendel (1921) no attempt was made by the former investigators to prevent the acid secreted by the stomach from reaching the intestinal mucosa. In consideration of this fact and as a result of other experiments, they could not concur in this opinion.

The association of appetite with vitamin B and its relation to gastric secretion has frequently been the subject of investigation. Lumière (1920) believes that the lack of appetite in pigeons on a polished rice diet is an expression of a lack of secretion of digestive juice, the gland not receiving a stimulus provided by vitamin B. Cowgill and Mendel (1921) re-investigated the alleged relationship of vitamin B as a stimulator of secretory glands. They failed to find any such action upon the salivary glands, the pancreas or the liver. Bickel (1922), working with dogs having a Pavlov pouch, concludes that the power of the gastric glands to secrete is not impaired by a vitamin-free diet, although diets lacking vitamins failed to stimulate secretion.

Failing to find a relationship between the vitamin B content of foods and the secretion of digestive glands, Cowgill, Deuel, Plummer and Messer (1924) adduce evidence of a dependence of gastric mobility upon this dietary factor. Dogs were placed on diets deficient in vitamin B and the character of the stomach contractions recorded by means of the balloon method of Carlson. In mild cases of vitamin B deficiency no remarkable change in the hunger contractions occurred. In serious cases a lack of tone, attended by a decrease in frequency, duration and amplitude of contractions was noticed. Finally the stomach became atonic. The administration of beef extract did not correct the gastric atony, but therapeutic doses of yeast vitamin concentrate or of a wheat germ extract "resulted in a characteristic prompt recovery of appetite, improvement in the general condition of the animal, and a return of the former normal contractility of the stomach" (p. 157).

The cases of polyneuritis columbarum which we have studied probably developed into a more severe type than that observed by Cowgill and his collaborators. Food was retained in the crop for some time prior to the onset of convulsions, a marked diarrhea occurred, and at autopsy atrophy of the pancreas and gastro-intestinal tract and fluid infiltration into the pericardial sac and peritoneal cavity were frequently noted.

EXPERIMENTAL WORK. The pigeons on the experimental diets described in our previous paper (Farmer and Redenbaugh, 1925) were used to obtain the data presented here. Of those developing polyneuritis, nos. 5, 6, 7, 8 and 12 were killed or died during convulsions, and the pancreas and first six inches of the intestine were promptly removed. As controls, we used nos. 4, 9, 14 and 19, all of which had served as controls in the previous investigation and had been continuously on an adequate diet. The pancreas and the first six inches of intestine were likewise removed from these birds. After quickly washing under the cold water tap, the pancreas and intestinal section of each bird were dried by wrapping loosely in a clean towel, then removed and weighed together. They were then finely hashed in a small food grinder, placed in a large test tube, and a quantity of glycerol equal to twice the weight of the hashed organs added. The mass, at first very thick, gradually became of a more fluid consistency as the result of autodigestion. The linings of the stomach of each pigeon were removed and preserved together in a similar manner.

After several weeks, depending upon the evident digestion, the glycerol extracts were centrifuged and 1 gram of clear supernatant fluid added to a small Erlenmeyer flask containing 49 grams of pure glycerol. After stoppering, the contents were thoroughly mixed by shaking. Enzyme preparations of this nature have been used by Vernon (1901) and by Long and Muhleman (1914). No decrease in activity is noticeable for several months.

It should be pointed out here that by the above method of preparation, the final glycerol extracts are representative of equivalent weights of the original organs taken. A comparative study of the enzymatic content or efficiency is therefore possible. It is of interest to mention that a marked difference in the autodigestion of material obtained from the normal and the polyneuritic pigeons was observed. The digestion of the former was practically complete, whereas the tubes containing the latter had a considerable quantity of tissue remaining at the time they were centrifuged.

Starch digestion. In determining the relative amylolytic power of the different pancreatic-intestinal extracts, the method of Long and Johnson (1913) was used. The results are given in table 1.

It will be seen that the extracts prepared from the pancreas and intestine of normal pigeons gave unmistakable evidence of digestion after 10 minutes' incubation. After 2 hours' incubation the starch cleavage had progressed

to a point where little or no color was produced from a dextrin, only the faint yellow of the dilute iodine solution remained. The extracts prepared from the pancreas and intestine of all polyneuritic pigeons, with the exception of no. 6, showed no evidence of digestive activity during 2 hours of incubation. We therefore believe that in severe polyneuritis the pancreas either ceases to form a starch-splitting enzyme or, if formed and secreted, it remains inactive due to the absence of an intestinal activator. Possibly both enzyme and activator are absent.

The cleavage of lipins. The influence of polyneuritis upon the digestive efficiency of pancreatic lipase was determined by the ethyl butyrate method. No attempt was made to distinguish a true lipase from an esterase, all evidence of cleavage being attributed to the former.

TABLE 1

Digestion of starch

(Incubation at 38°C.)

PIGEON NUMBER	TIME						
	5 minutes	10 minutes.	20 minutes	30 minutes	50 minutes	80 minutes	120 minutes
Normal pigeons							
4	Blue	Blue	Purple	Purplish red	Red	Light red	Faint yellow
9	Blue	Purple	Purplish red	Purplish red	Red	Red	Faint yellow
19	Blue	Purple	Purplish red	Purplish red	Red	Light red	Faint yellow
Polyneuritic pigeons							
6	Blue	Blue	Blue	Blue	Blue	Blue	Purple
7	Blue	Blue	Blue	Blue	Blue	Blue	Blue
8	Blue	Blue	Blue	Blue	Blue	Blue	Blue
12	Blue	Blue	Blue	Blue	Blue	Blue	Blue

The determination, as made, consisted in adding 5 cc. of the glycerol extract of the pancreas and intestine to 50 cc. of a saturated aqueous solution of ethyl butyrate. After incubating for two hours at 38°C., 25 cc. of the digestion mixture were removed, 25 cc. of neutral alcohol and 5 cc. of neutral ether were added and the mixture then titrated with 0.1 N alkali, using phenolphthalein as the indicator. Control determinations with boiled glycerol extract were run on each enzyme preparation. Table 2 gives the data obtained with extracts prepared from both the normal and the polyneuritic pigeons.

As seen from table 2, the polyneuritic pigeons suffer a complete inability to cleave a fatty acid ester. This we believe is evidence of the failure of

the pancreas to form a lypolytic enzyme, or if formed and secreted, that it remains unactivated.

TABLE 2

Cleavage of ethyl butyrate

PIGEON NUMBER	TITRATION—CC. 0.1 N ALKALI	CONTROL—CC. 0.1 N ALKALI	FATTY ACID FORMED—CC. 0.1 N
Normal pigeons			
4	1.10	0.50	0.60
9	1.00	0.45	0.55
14	1.20	0.50	0.70
19	1.20	0.50	0.70
Group average			0.64
Polyneuritic pigeons			
6	0.45	0.40	0.05
7	0.50	0.50	0.00
8	0.50	0.50	0.00
12	0.45	0.40	0.05
Group average			0.025

TABLE 3

Digestion of casein

(Cubic centimeters of 0.1 N sodium hydroxide)

PIGEON NUMBER	TOTAL TITRATION	TITRATION OF CONTROL (ENZYME BOILED)	EQUIVALENT OF DIGESTION
Normal pigeons			
4	11.10	3.70	7.40
9	9.30	3.70	5.60
14	9.10	3.70	5.40
19	8.90	3.60	5.30
Group average			5.95
Polyneuritic pigeons			
5	6.80	3.60	3.20
6	7.80	3.60	4.20
7	7.20	3.60	3.60
8	7.30	3.60	3.70
12	6.40	3.60	2.80
Group average			3.50

Protein digestion. To determine the proteolytic activity of the extract prepared from the pancreas and intestine of normal and polyneuritic pigeons, 1 gram of the clear glycerol extract obtained by centrifuging the

autolysed mixture as described above, was added to a flask containing 50 cc. of 4 per cent casein solution. The latter was prepared from highly purified material and contained 40 grams of casein and 340 cc. of 0.1 N sodium hydroxide per liter. After the addition of a drop or two of toluol, the flasks were incubated at 38°C. for 2 hours. At the end of this period, the relative amount of protein cleavage effected by the various enzyme extracts was determined by means of the formol titration method of Sörensen as modified by Long and Barton (1914). The results are given in table 3.

The figures in the above table clearly indicate a marked decrease to occur in the proteolytic activity of the glycerol extracts obtained from the pancreas and intestine of the polyneuritic pigeons. In contrast to the absolute lack of digestive power exhibited when testing for starch and fat cleavage, protein digestion remains at nearly 60 per cent of its normal efficiency.

Milk coagulation by the stomach extract. As previously mentioned, a glycerol extract was prepared from the linings of the stomachs of the

TABLE 4

Milk coagulation by a gastric ferment

	NORMAL PIGEONS				POLYNEURITIC PIGEONS			
	No. 4	No. 9	No. 14	No. 19	No. 6	No. 7	No. 8	No. 12
Coagulation time (minutes)..	5	12	8	14	30	35	35	45
Group average.....	9.35 minutes				34 minutes			

normal and polyneuritic pigeons. In the material from the normal pigeons we were able to demonstrate the presence of a rennin-like substance, which although present in the extracts from the polyneuritic pigeons, exhibited a marked decrease in coagulability. In determining the efficiency of these preparations, 1 drop of each extract was added to 5 cc. of milk in a test tube, after which it was placed in an incubator at 38°C. until coagulation occurred. The data are presented in table 4.

SUMMARY AND CONCLUSIONS

Glycerol extracts were prepared from the pancreas and first six inches of intestine of normal pigeons and of pigeons kept on diets deficient in vitamin B until a severe polyneuritis developed. Similar extracts were prepared from the stomachs of each pigeon. The extracts from the pancreas and intestine were used in digestion experiments with starch, ethyl butyrate and casein as substrates respectively.

Efficient digestion occurred in all cases where the extracts of normal pigeons were used. When using the extracts obtained from the poly-

neuritic pigeons, no digestion of starch or ethyl butyrate occurred. The digestion of casein on the contrary was about 60 per cent of that observed in the normal pigeons. Evidence is produced of a similar decrease in efficiency of a rennin-like enzyme occurring in the stomachs of the pigeon.

We believe that the experiments cited here clearly indicate that in severe polyneuritis columbarum a marked decrease occurs in the digestion of starch, fat and protein; the digestion of starch and fat becoming nil if the condition reaches the degree of severity marked by convulsions. This failure to digest starch and fat we believe to indicate that the pancreas either ceases to form amylolytic and lipolytic enzymes or, if formed and secreted, they remain inactive due to the absence of an intestinal activator.

We furthermore believe that the evidence presented here justifies our inference as stated at the beginning of this paper; that during recovery, the influence of vitamin B must at least in part be directed at a restoration of function of the enzymes of digestion, as well as of those of tissue oxidation.

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A COMPARISON OF THE IRRITABILITY OF MEN AND WOMEN

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It is generally conceded that men are less irritable than women, even in the absence of any definite proof as a basis for such an opinion except, perhaps, casual observation. It has been suggested that the condition of irritability in women follows somewhat closely the various stages of the menstrual cycle. This question has been studied by a number of investigators, among them King (1918) and more recently Tuttle (1925). The former found that a period of hyper-irritability precedes or accompanies the onset of menstruation, followed by a decline which continues for a few days after the menses has ceased and then there is a slight rise during the intermenstrual period. The latter found that in some cases there is no significant change in irritability during the menstrual cycle, in others there may be depressed irritability during and preceding this period, while in still others hyper-irritability may precede, continue during and follow the menstrual period. Although the work of King and Tuttle is not conclusive as to the variation in irritability during the menstrual period, their work indicates that there is a variation during this cycle.

In view of the paucity of data on the question of the relative irritability of men and women, the writer carried out the investigation herein reported. Since the knee-jerk has been shown to be a reliable index to the general tone and irritability of the body, it was used to secure the data presented here. The knee-jerk is now used as a diagnostic indication in lesions of the cord, particularly for the lumbar region. In the disease known as locomotor ataxia the tracts of Goll and Burdach are affected and as a result the jerk is diminished or abolished altogether according to the stage of the disease. So, also, lesions such as occur in infantile paralysis which affect the anterior horns of the gray matter, destroy the reflex by cutting off the motor paths, while in other cases lesions affecting the pyramidal system may be accompanied by an exaggeration of the jerk. According to results obtained up to the present time, it is reasonable to suppose that any variation in the general bodily tone or irritability will be exhibited by a comparison of the knee-jerks of any group of subjects. This reflex has been studied by a number of investigators who pointed out that, if it is used as a quantitative measure, a number of factors must be carefully considered in securing the data.

While studying the knee-jerk, Lombard (1887) found that not only did it show a wide diurnal variation, but that it was also influenced by exercise, cold baths, food, the strength of blow used in eliciting it, music and mental activity. Carlson (1916) observed that hunger contractions frequently increase the reflex response of the cord to such an extent that a standard minimal stimulus causes a maximal response. He observed also that the reflex excitability usually falls to normal during the pauses between the single contraction, and after the strong hunger period it appears to be somewhat lower than normal. He further observed that a stimulation of the gastric mucosa by hot or cold water introduced into the stomach by a tube, thus not stimulating the mouth or esophagus, invariably augments the knee-jerk in man.

Dodge (1911) and recently Tuttle (1925a) made similar observations with reference to the strength of the blow used to elicit the jerk. It was found that not only was it necessary to use a stimulus of constant intensity but the position where the stimulus impinges was also an important factor. These investigators also showed that in studying the normal knee-jerk a stimulus should be used which is submaximal, in fact, one not far above the threshold of response. While studying a large number of cases, it was found that 50 grams was the most suitable for the majority of subjects and thus it was selected as the strength of stimulus in this investigation.

As far as the writer has been able to find, there are no data available with reference to the variation due to the age of subjects. In order to eliminate this as a possible source of error, age limits were considered.

The attention of a subject while making a record has been shown by Tuttle (1924) to increase the extent of the knee-jerk very materially, depending upon the degree of attention. For this reason conditions must be kept as uniform as possible while records are being made. The writer observed that unfamiliarity with the apparatus and the general surroundings were distracting factors and must be considered.

The activity of the subject previous to the experimental period is important. Lombard found that exercise such as stair climbing lessened the extent of the knee-jerk while Sternberg (1887) concluded, after carrying out a similar investigation, that an increase in the extent of this reflex was an indication of fatigue. Recently Brown (1925) while making a study of the effect of exercise on the knee-jerk, found that in some cases there was a marked increase while in others a decided decrease, and concludes that the extent of the knee-jerk depends upon the physical condition of the individual subject.

Mitchell and Lewis (1886), in studying the factors which increase and those which lessen the knee-jerk, observed a reinforcement consequent not only upon voluntary acts, but upon painful stimulation of the nerves of the skin either by pinching or by application of heat, cold, or electricity.

A similar reinforcement was noticed when the eyes were exposed to the light of burning magnesium wire.

In securing data of the nature presented in this paper, temperature and drafts of air have been shown to be of significant importance. Bowditch and Warren (1890) found that air from an electric fan augments the knee-jerk. Recently Emery (1925) not only confirmed this point but showed that a change in temperature is a determining factor in knee-jerk records.

The data were collected under the following conditions:

a. A stimulus of 50 grams was used.

b. The stimuli were delivered to the center of the ligamentum patellae of the right knee.

c. The records were made between 10 and 12 a.m.

d. If unusual exercise was reported the subject was dismissed.

e. The limit of age was placed between 18 and 24 years.

f. Only normal subjects were used.

g. In case of women the records were made during the "period of rest," approximately the middle of the period (Howell, 1924).

h. Environmental conditions were kept as constant as possible in an investigation of this nature.

i. The length of sitting for permanent records was 15 minutes in each case, the hammer striking the patellar tendon 7 times a minute, thus giving an approximately constant number of kicks to be recorded for each subject.

j. The temperature was kept constant and all air drafts prevented.

The point of impingement of the stimuli was determined by palpating the leg (ligamentum patellae). Although it is probable that a slight error is ever present due to variation in position, it was found that with practice one was able to locate the center of the tendon accurately. The age limit was fixed between 18 and 24 years because of the fact that students must be used as subjects and it was found that there were the greatest number available between these ages.

A subject, who reported that he had not experienced or was not experiencing at the time of the experiment any unusual symptoms of disease, was considered a normal subject. The subjects were adjusted in the apparatus and left alone during the time the records were made, so in each case the environmental stimuli, were practically constant.

The susceptibility of the knee-jerk to the environmental conditions and experimental error makes it desirable to employ an apparatus for eliciting it which automatically delivers uniform stimuli of equal intensity to the desired position. Such an apparatus has been described by Tuttle (1924a) and was used for securing the data recorded in this paper. The forward component of the excursion of the subject's foot is used as an index of the extent of the knee-jerk which is recorded as the distance through which the

stylus moves. This distance is referred to as the "height" or "extent" of the knee-jerk. The records read from right to left due to the fact that the base line is at the top when the records are made.

TABLE 1

MEN		WOMEN		MEN		WOMEN	
Subject	Height of kick	Subject	Height of kick	Subject	Height of kick	Subject	Height of kick
	mm.		*mm.*		*mm.*		*mm.*
17	76.65	48	66.34	60	7.29	39	14.85
64	43.94	27	58.69	51	7.25	9	14.61
49	40.82	25	56.19	55	6.98	55	14.49
21	37.77	18	55.28	48	6.29	21	14.38
54	31.44	37	54.54	23	5.81	52	12.72
23	31.40	2	49.00	41	5.57	15	12.59
2	30.92	69	42.61	65	5.31	75	11.93
3	30.48	53	39.30	59	5.26	43	11.52
63	28.43	19	37.43	31	4.37	41	10.86
16	25.13	7	36.55	68	4.27	60	10.74
6	24.32	50	36.35	45	4.24	70	9.66
20	24.07	56	35.66	66	3.06	34	9.45
13	23.95	60	32.08	44	2.94	30	9.45
57	23.79	44	31.71	32	1.80	31	9.43
52	22.72	3	31.55	24	1.03	23	9.26
62	21.50	40	30.95	27	1.00	29	8.16
8	21.35	76	29.29	29	0.60	45	8.04
12	20.13	51	28.77	22	0.53	67	6.92
25	19.98	62	26.36	35	0.45	68	6.79
46	19.84	6	25.82	30	0.39	11	6.79
67	18.75	64	25.73	5	0.18	16	6.46
10	17.77	42	25.64	37	0.15	26	6.35
7	17.58	36	23.80	28	0.13	47	5.79
33	17.43	32	22.67	61	0.00	4	5.66
53	17.02	59	22.10	36	0.00	12	4.03
42	14.67	17	21.59	34	0.00	24	2.92
43	13.22	54	20.10	26	0.00	65	2.84
15	12.94	58	19.35	18	0.00	38	2.81
56	11.91	1	18.92			20	1.81
38	11.90	49	18.78			10	1.63
50	10.57	5	18.45			71	1.30
39	9.50	13	18.45			74	0.64
58	9.00	66	17.33			8	0.54
4	8.94	72	15.69			28	0.49
14	8.33	63	15.12			14	0.00

A fifteen-minute sitting of a subject was decided upon since our experience has shown that during a period of this length a group of kicks characteristic of a subject is delivered.

Data were collected from 63 men and 70 women. Table 1 shows the

TABLE 2

GROUP	HEIGHT OF KICK	NUMBER OF MEN	PERCENTAGE OF MEN	NUMBER OF WOMEN	PERCENTAGE OF WOMEN
	mm.				
A	0–9	32	50.79	25	35.72
B	10–19	13	36.51	18	41.43
	20–29	10		11	
C	30–39	5	12.70	9	22.85
	40–49	2		2	
	50–59	0		4	
	60–69	1		1	

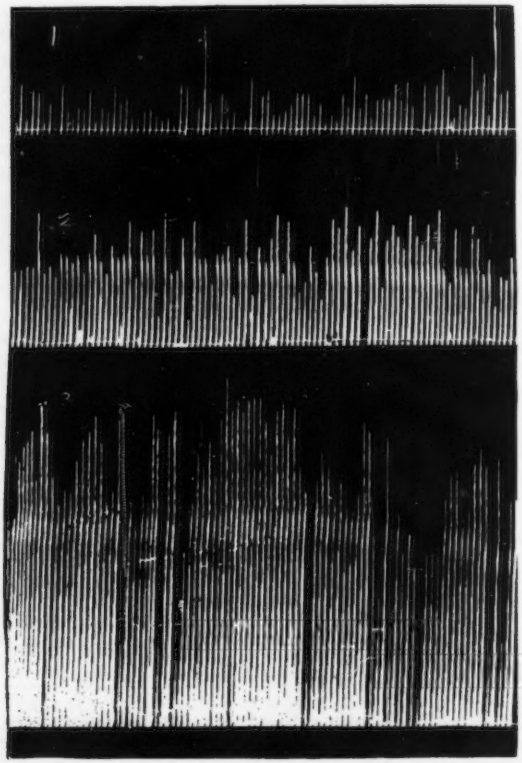

Fig. 1. Records characteristic of groups shown in table 2. *1*, median of group A; *2*, median of group B; *3*, median of group C.

average height of the knee-jerk of both men and women used in this investigation. Each subject delivered approximately 100 kicks.

Table 1 shows that in general the height of the kicks of the women studied is 42.08 per cent higher than those of the men. The average height kicked by the men is 13.71 mm.; by the women 19.48 mm.

From table 1 it is seen that the data fall into three natural groups as follows: *A*, those kicking less than 10 mm., *B*, those kicking more than 10 mm. but less than 30 mm., *C*, those kicking more than 30 mm. but less than 70 mm. This is shown in table 2.

Figure 1 shows the records of the subjects whose average knee-jerk is the median of the group under which the data are presented in table 2.

Table 2 shows that for men 50.79 per cent fall in group A, 36.51 per cent in group B and 12.70 per cent in group C. In general the same is true for women. Of the entire number of women studied 35.72 per cent fall in group A, 41.43 per cent in group B and 22.85 per cent in group C. In group A there are 7 more men than women and in group B there are 6 less men than women, while in group C there are twice as many women as men. While the highest average kick is found among the men, five were found who showed no kick with the given stimulus, while only one woman falls in this group. It was found, however, that if the intensity of the stimulus was increased sufficiently, each of these subjects responded with a kick. Taken as a group or according to sex grouping, approximately 80 per cent of the subjects are found in groups A and B leaving only 20 per cent in group C. There are practically the same number of men and women in groups A and B, but there are twice as many women as men in group C.

SUMMARY

The extent of the knee-jerk of 63 men and 70 women was compared. It was found that the average height kicked by the men was 13.71 mm. while for the women it was 19.48 mm. thus showing that the knee-jerk of the women used in this investigation is 42.08 per cent higher than that of the men.

The percentage distribution of subjects according to the height of the knee-jerk shows that 50.79 per cent of the men are low kickers, 36.51 per cent are medium, and 12.70 per cent are high. In the case of women 35.72 per cent are low, 41.43 per cent are medium, and 22.85 per cent are high.

It was also found that the knee-jerk was absent much more frequently in men than women.

Assuming that the knee-jerk is an index to irritability, the data show that women are perceptibly more irritable than men.

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THE EFFECT OF ULTRA-VIOLET RADIATION UPON EXPERIMENTAL TETANY[1]

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In 1919 Huldschinsky (1) first reported the interesting discovery that the ultra-violet ray exerted a curative action in rickets. As a result of his investigation, a great deal of attention has been devoted to the relation of ultra-violet rays to calcium and phosphorus metabolism. Since rickets is associated with defective calcium and phosphorus metabolism and is accompanied by tetany in a considerable number of cases, a syndrome also intimately connected with, if not due to faulty calcium metabolism, it is not surprising that some attention should have been paid to the effect of ultra-violet rays upon infantile tetany. Accordingly, very soon after the publication of Huldschinsky's (1) paper, Sachs (2) reported that treatment with the ultra-violet ray cured latent tetany in infants.

Sachs' results are of considerable interest. He worked with seven children presenting symptoms of latent tetany and also active rickets. The tetany was diagnosed by the usual methods, i.e., the lowered threshold of the peripheral nerves to mechanical and electrical stimulation (signs of Chevostek and Erb). Before and during the experiment none of the patients received any calcium or phosphorus. The children were exposed to the ultra-violet ray for varying periods and for varying lengths of time each day, but the longest exposures were 20 to 25 minutes. All of the symptoms of tetany disappeared after a few treatments.

Huldschinsky (3) within a few months following publication of the paper by Sachs, announced that he was able to cure manifest tetany in children by the ultra-violet ray. In the six cases of infantile tetany (complicated by rickets) studied, the symptoms, such as mechanical and electrical excitability of the peripheral nerves, laryngospasm and muscle cramps, disappeared within a short time following radiation. The laryngospasm and cramps usually responded to the first intensive radiation. Latent symptoms such as the Chevostek and Erb signs, required longer treatment.

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Sachs (4) was also able to cure manifest tetany of infants by ultra-violet rays.

So far as the present writers are aware, the reports of Huldschinsky and Sachs are the only accounts in the literature dealing with the effect of ultra-violet rays upon the tetany syndrome. We have been unable to find any reports of the effect of this physical agent upon experimental tetany following parathyroid removal. Since investigators are not agreed that infantile tetany and experimental tetany are identical syndromes, or that both are due to destruction or lesions of the parathyroid glands, it was considered worthwhile to test the effect of ultra-violet radiation upon parathyroidectomized dogs. Ten adult dogs were used, nine of which were males. The animals were short-haired and white, except two which were light tan in color. Pure white animals were difficult to obtain, most of our animals had one or two splotches of brown or black scattered over the body. Special attention was devoted to the food, and we employed the diet recommended by Cowgill (5) with slight modification. The constituents of the diet are as follows:

	GRAMS	CALORIES	PER CENT
Casein (commercial)	6.3	21.2	41.2
Sucrose	4.5	18.8	29.4
Lard	2.8	26.5	18.3
Butter	1.1	9.5	7.2
Bone ash (omitted)	0.4		2.6
Salt mixture (modified)	0.2		1.3
	15.3	76.0	100.0

The salt mixture used by Cowgill contains the following:

	grams
Sodium chloride	10.0
Calcium lactate	4.0
Magnesium citrate	4.0
Ferric citrate	1.0
Potassium iodide	0.1

We omitted the calcium lactate from the salt mixture and the bone ash from the diet because of the calcium. However, it may be said that experiments on animals have shown that the addition of bone ash to the diet has no preventive action on tetany, nor is the onset of the symptoms at all delayed in those animals receiving it in considerable quantity. Berkeley and Beebe (6) emphatically state that the addition of bone ash to the food of parathyroidectomized dogs has no effect upon the resulting tetany. An adequate supply of vitamin B was obtained by using prepared

yeast tablets (Harris) and crumpling them in the food. Each dog received approximately 16 grams of the diet per kilo body weight each day.

The animals were radiated with a quartz mercury vapor lamp with a spectral energy distribution of approximately 30 per cent ultra-violet; 50 per cent visible and 20 per cent infra red (10). The distance between the lamp and the dog's back was 40 cm. when the animals were in a prone position, but since they sat or stood much of the time, the distance was considerably less. The animals were placed in wooden boxes with the top and one side removed and replaced by coarse meshed wire. It was soon found necessary to keep the operated animals cool while under the light, otherwise they rapidly develop violet tetany, the heat apparently serving to induce manifest symptoms. An electric fan was used to cool the animals while being radiated.

We are greatly indebted to Prof. Henry Laurens of the Department of Physiology for use of the light.

Before operation the dogs were radiated one hour per day for several days. It was thought that such treatment might raise the level of the serum calcium and so prevent tetany from developing. Grant and Gates (7) reported a rise in serum calcium in rabbits following exposure to ultra-violet rays, and the marked effect of such radiation upon the calcium and phosphorus metabolism in rickets is well known. However, it is evident from examination of table 1 that one hour a day exposure is not sufficient to materially affect the level of the serum calcium of normal dogs. Clark and Collip's (8) modification of Kramer and Tisdall's (9) method for calcium determination was employed. Most of the samples were run in triplicate. The blood was taken directly from the heart since our experience has been that this is the simplest and least painful method for bleeding dogs. We have never had any infections or noted any ill effects from repeated heart punctures.

Several weeks after we had started the experiments, a paper appeared by Mayerson, Gunther and Laurens (10) in which they showed that radiation for one hour a day at 40 cm. for eight days is not sufficient to raise the serum calcium of the blood. In fact, such radiation served to increase the phosphorus and correspondingly decrease the calcium during treatment. However, single exposures of two hours for eight days caused both calcium and phosphorus to show about parallel curves, a rise in phosphorus being accompanied by a simultaneous increase in calcium.

The data presented in table 1 show that in four cases, dogs 4, 5, 6 and 7, the serum calcium was slightly lower after radiation of one hour a day for periods varying from five to ten days, than it was before. No efforts were made to determine the phosphorus. We quickly noted that following removal of the parathyroids, the radiated dogs developed tetany as rapidly

as non-radiated dogs. In none of our cases did we observe any evidence that an hour a day exposure to ultra-violet rays, for periods varying from four to twelve days, exerted any effect in retarding the onset of tetany. This fact is even more clearly indicated in table 2. For instance, dog 1

TABLE 1

Effect of radiation upon the serum calcium of normal dogs

ANIMAL	SEX	WEIGHT	HOURS PER DAY RADIATED	DAYS RADIATED BEFORE OPERATION	Ca BEFORE RADIATION	Ca AFTER RADIATION BUT BEFORE OPERATION	REMARKS
		kilos			*mgm.*	*mgm.*	
1	♀	4	1	4	10.5	11	Animal pregnant
2	♂	7	1	12	10.5	11.1	Severe conjunctivitis
3	♂	6	1	9	10.1	10.8	Slight conjunctivitis
4	♂	4	1	6	10.0	9.5	Slight conjunctivitis
5	♂	4	1	10	11.4	11.1	Severe conjunctivitis
6	♂	3	1	6	12.0	10.6	
7	♂	5	1	5	12.2	12.1	

TABLE 2

The effect of radiation upon the serum calcium of parathyroidectomized dogs

ANIMAL	HOURS RADIATED BEFORE OPERATION	DATE OPERATED	Ca BEFORE OPERATION	DATE TETANY APPEARED	Ca AFTER TETANY APPEARED	INTERVAL BETWEEN OPERATION AND APPEARANCE TETANY
			mgm.		*mgm.*	
1	4	May 12	11.0	May 16	6.6	4 days
2	12	June 12	11.1	June 15	6.5	3 days
3	9	June 18	10.8	June 20	7.6	2 days
4	6	June 18	9.5	June 21	7.0	3 days
5	10	June 21	11.1	June 22	5.5	1 day
6	6	June 18	10.6	June 20	6.7	2 days
7	5	May 12	12.1	May 14	7.2	2 days
8	3	May 12	Not determined	May 15	Not determined	3 days
9	5	April 28	Not determined	April 28	Not determined	6 hours
10	4	July 6	Not determined	July 8	Not determined	2 days

was radiated four hours before the operation and tetany appeared on the fourth day following parathyroidectomy. Dog 2 was radiated twelve hours before operation and tetany developed on the third day after parathyroid removal. Dog 5 was radiated one hour a day for ten days before operation and showed violent tetany within twenty-four hours after para-

thyroidectomy. It is quite clear that radiation of one hour a day for one or two weeks does not prevent or retard the appearance of tetany in dogs.

Several investigators have noted that the onset of tetany coincides with a marked diminution in the serum calcium from about 10 or 11 mgm. per 100 cc. to approximately 7 mgm. or below. The figures presented in table 2 are of interest in this connection since they afford an interesting confirmation of this observation. Dog 3 is exceptional in that the serum calcium was 7.6 mgm. per 100 cc. (triplicate determination) ten minutes after the first symptoms appeared. (See protocol of dog 3.) The animal had shown no symptoms whatever until 2 p.m. of June 20 when he suddenly lost control of his left hind leg and began running around in circles, yelping

TABLE 3

The effect of radiation upon the life span of parathyroidectomized dogs

ANIMAL	HOURS RADIATED BEFORE OPERATION	HOURS RADIATED AFTER OPERATION	TOTAL NUMBER OF HOURS RADIATED	DURATION OF EXPERIMENT AFTER PARATHYROIDECTOMY	REMARKS
				days	
1	4	23	27	23	Died of malnutrition and exhaustion
2	12	36	44	16	Died of tetany
3	9	53	62	25	Died of malnutrition and exhaustion
4	6	14	20	5	Died of tetany and pneumonia
5	10	25	35	8	Died of tetany
6	6	6	12	2	Died of tetany convulsions upon passage of stomach tube
7	5	3	8	2	Died in convulsions upon being prepared for bleeding
8	3	9	12	6	Died in convulsions
9	5	13	18	9	Died of convulsions
10	4	33	37	18	Died of malnutrition and exhaustion

and panting. He was immediately bled and the calcium determinations made. The remaining animals listed all showed a marked fall in the level of the serum calcium upon the appearance of tetany.

Examination of table 3 shows that prolonged radiation of parathyroidectomized dogs greatly lengthens the life span of such animals. It has been our experience, based upon observations of sixty parathyroidectomized dogs, that very seldom does a dog survive longer than four or five days following the appearance of typical tetany symptoms. The syndrome in cats is somewhat less violent and occasionally these animals may live a week or ten days after removal of the parathyroids (11); but, as stated before, the situation is otherwise in dogs. Animal 1 lived twenty-three days after the first signs of tetany appeared, and during this time was seldom entirely

free from symptoms but remained in a tetanoid condition, i.e., was quiet and slept most of the time, but if aroused suddenly, or handled roughly, immediately went into epileptiform convulsions. This same tetanoid state was exhibited by all radiated dogs which survived over ten days.

The ameliorative action of ultra-violet radiation upon tetany is sometimes very striking. Many times we have observed dogs presenting violent tetany before exposure to ultraviolet rays, quiet down and return to a condition apparently normal after three hours' radiation. (See protocols of animals 1, 2 and 3.) It is a fact known to all investigators of experimental tetany, that dogs presenting alarming symptoms may temporarily recover and for a few hours seem normal, only later to die in convulsions or from exhaustion. The animal may show several such remissions before death supervenes, so that one must be cautious about attributing too much curative power to any agent which apparently induces a temporary subsidence of symptoms, because the return to normal may be spontaneous on the part of the animal and not due to the agent regarded as responsible for the cure. However, we have seen cessation of violent tetany following prolonged radiation so frequently and consistently, that we are convinced that ultra-violet rays exert a marked ameliorative effect upon experimental tetany. As shown in the protocols (especially dogs 2 and 3), the effects of radiation upon manifest symptoms are striking. In none of our cases has prolonged radiation completely cured the animals; the manifest symptoms may disappear for a few days or hours to return later and then again disappear following radiation. Ultra-violet radiation is not sufficient (in the exposure employed by us) to completely restore the animal to normal after parathyroidectomy. Such radiation is, however, efficacious in keeping the animal alive a sufficient length of time to permit it to adjust itself to the lowered blood calcium, and the dog passes into the tetanoid state mentioned previously, in which it remains in a drowsy, depressed condition until death from exhaustion or malnutrition occurs.

Radiated, parathyroidectomized dogs do not eat voluntarily, and must be given food by stomach tube. Despite the fact that our operated animals were daily fed a ration sufficient to keep a normal dog in excellent condition, they rapidly lost weight and became greatly emaciated. The animals had difficulty in holding their food and vomited much of it, and of the part retained not all was utilized. Several times it was noted at autopsy that large lumps of undigested casein remained in the stomach ten or twelve hours after feeding. Undoubtedly, the immediate cause of death in three of the radiated dogs (1, 3 and 10) was starvation and not tetany. Several dogs (animals 4, 6 and 7—table 3) developed violent tetany and died within a few days, despite radiation. Dogs 6 and 7 lived but two days following the appearance of typical tetany. Both animals died in convulsions. In both instances the convulsions were induced by handling the animal while

in tetany. Neither animal afforded a fair test of the efficacy of ultra-violet radiation in alleviating tetany symptoms.

Table 4 shows the effect of radiation upon the serum calcium of tetany dogs. It will be noted at once that, despite the amelioration of violent symptoms, the level of the serum calcium was not greatly affected. Following the appearance of manifest tetany the serum calcium was

TABLE 4

The effect of prolonged radiation upon the serum calcium of parathyroidectomized dogs

ANIMAL	DATE OF OPERATION	SERUM Ca	SERUM Ca	SERUM Ca	SERUM Ca
1	May 12	May 12, 11 mgm.	May 18, Tetany, 6.6 mgm.	May 24, Tetanoid condition, 5.5 mgm.	June 1, Tetanoid condition, 4.4 mgm.
2	June 12	June 12, 11 mgm.	June 15, Tetany 6.5 mgm.	June 23, Tetanoid condition, 4.9 mgm.	
3	June 18	June 18, 10.8 mgm.	June 20, Tetany, 7.6 mgm.	June 22, Violent tetany, 5.1 mgm.	June 26, Violent tetany, 6 mgm.
4	June 18	June 18, 10 mgm.	June 21, Mild tetany, 7 mgm.		
5	June 21	June 21, 11.1 mgm.	June 24, Tetany, 5.5 mgm.	June 28, Mild tetany, 5.9 mgm.	
6	June 18	June 18, 10.6 mgm.	June 20, Tetany, 6.7 mgm.		
7	May 12	May 14, 12.1 mgm.	June 14, Violent tetany, 7.2 mgm.		

generally reduced to below 7 mgm. per 100 cc. and remained below this level despite the prolonged radiation. In no case was the serum calcium raised to anything approximating normal, i.e., 10 or 11 mgm. per 100 cc. of blood. The animals once safely past the critical period of the first five or six days seemed to be able to adjust themselves to the low blood calcium, because after this period the dogs which survived passed into the tetanoid condition characterized by greatly lowered blood calcium. Animal 1

(table 4) at the end of twenty-three days after parathyroidectomy showed an extremely low serum calcium, 4.6 mgm., and yet active violent symptoms were absent, the animal dying from malnutrition. Such a low blood calcium if found in a newly operated dog would in all probability have been accompanied by violent tetany. Salvesen (12) has reported that parathyroidectomized dogs can be kept alive for long periods on a milk diet alone, despite the lowered blood calcium, and that such animals adjust themselves to the diminished calcium of their blood and tissues.

It is obvious from our data that prolonged radiation with ultra-violet is much less efficacious in alleviating the symptoms of tetania parathyropriva in dogs than it is in the treatment of infantile tetany, according to the reports of Sachs (2) and Huldschinsky (3). Radiation does greatly prolong the lives of some of the operated dogs (table 3), but in none of our cases was a permanent cure effected. The violent symptoms could be held in check and in some cases completely controlled, but the animal passed into a tetanoid condition, finally dying of malnutrition or exhaustion. One explanation for this difference in the results of radiation upon infantile tetany and experimental tetany which suggests itself, is the fact that dogs react to parathyroid removal quickly and violently, much more so than do humans and many other mammals. Experimental tetany of dogs and manifest infantile tetany differ considerably, in most cases, in regard to the degree of violence exhibited. Parathyroid removal in dogs is almost invariably fatal except in a few anomalous instances where accessory glands are probably present which were not removed at operation. On the other hand, infantile tetany is not by any means invariably fatal. In dogs, the tetany following gland extirpation is of such a violent character that the amount of radiation employed by us was not quite sufficient to completely allay the symptoms by raising the blood calcium to a normal level.

The point of interest, aside from the alleviation or cure of tetany by means of ultra-violet radiation, is the means by which this is accomplished. It is evident from the work on rickets, experimental tetany, and recent investigations upon the effect of ultra-violet rays on calcium and phosphorus metabolism, that any preventive or curative action ultra-violet rays may exert upon tetany is probably a result of altered calcium metabolism. Orr, Holt, Wilkins and Boone (13) report that ultra-violet therapy promotes the absorption of calcium and phosphorus from the intestines. They found that in infants with active rickets there was little if any retention of calcium and phosphorus in the body, and that most of the ingested phosphorus and almost all of the calcium was to be found in the stools. After treatment with ultra-violet rays, however, coincident with the healing of the rachitic lesions there occurred a greatly increased retention of calcium and phosphorus, so that one-third to one-half of these elements was retained.

It appears probable, therefore, that the curative action of radiation upon both infantile and experimental tetany is due to the fact that calcium absorption is promoted from the intestines, and the absorbed calcium obtained from the food is retained for the most part. Our data, however, do not show that the level of the blood calcium is raised to any appreciable degree by prolonged radiation of operated animals despite the fact that such treatment may greatly prolong life.

In infantile tetany the parathyroids as a rule present no specific lesions but, despite this, the blood calcium is greatly diminished (14). Grant and Gates (15) have shown that radiation of normal rabbits with ultra-violet rays induces hypertrophy of the external parathyroid gland, the size increase amounting in some instances to more than half the normal weight. These same investigators state that the greatest increase of serum calcium occurs during the period of progressive gland hypertrophy, and falls to normal during the period when the parathyroids are undergoing regression.

It is possible that another reason for the more striking effects of ultra-violet radiation upon infantile tetany than on experimental tetany is the direct stimulation of the parathyroid tissue by the rays. Protocols of three radiated dogs are given in abbreviated form below.

Protocol, dog 1. Female, white, short-haired, weight 4 kilos. Animal pregnant. Radiated 1 hour a day for four days before operation. Parathyroidectomy, May 12. Radiated 2 to 3 hours a day except when stated in the protocol.

May 13–15. Animal normal.
May 16. Tetany. Violent convulsions developed while dog was being radiated. Recovered after 3 hours' radiation.
May 17. Violent tetany. Symptoms abated after radiation.
May 18. Mild tetany. Serum calcium 6.6 mgm. per 100 cc.
May 19. Mild tetany. Symptoms disappeared following radiation.
May 20. Animal losing weight but has only mild tetany symptoms.
May 21–23. Slight tremors of leg and shoulder muscles.
May 24. No tetany symptoms, animal sleeps continually; serum calcium 5.5 mgm.
May 25–27. Animal weak but shows no tetany symptoms.
May 27–29. Animal not radiated; no symptoms, sleeps continually; fed by stomach tube.
May 29–31 Dog not radiated; no tetany; animal very weak.
June 1. No manifest tetany symptoms. Dog went into convulsions when stomach tube passed. Revived with difficulty. Serum calcium 4.4 mgm. Animal had convulsions when strapped to the table for bleeding. Animal quiet and does not show symptoms unless handled or frightened.
June 2. Slight tetany symptoms. Radiated 3 hours, first radiation since May 27.
June 3–4. Very mild tetany symptoms.
June 5. Very mild symptoms. Animal died in convulsions upon passage of stomach tube. Autopsy showed animal in advanced pregnancy.

Protocol, dog 2. Adult, male, white, short-haired, weight 7 kilos. Radiated 1 hour a day for 12 days; parathyroidectomized.

June 12. Serum calcium of dog before radiation 10.5 mgm. per 100 cc., after twelve days' radiation, serum calcium 11.1 mgm. Radiated 3 to 4 hours a day throughout the experiment.
June 12–14. Dog showed no symptoms. Radiated.
June 15. Tetany, very violent symptoms. Serum calcium 6.5 mgm. Violent symptoms disappeared after radiation; animal quiet.
June 16–17. No tetany. Animal sleeps most of the time.
June 18. Slight tremors.
June 19–21. No tetany. Faint tremors occasionally appear. Dog fell down a flight of three steps while running about and passed into an epileptiform convulsion. Revived by artificial respiration.
June 22. Animal in good condition, runs about in lively fashion but if startled or handled roughly will pass into convulsions.
June 23. Dog in good condition. Serum calcium 4.9 mgm. Animal sleeps most of the day. Fed by stomach tube.
June 24–26. Dog in good condition. No tetany symptoms if allowed to remain quiet. Convulsions if startled or handled roughly.
June 27. Dog in good condition. Walks about in normal fashion but when pushed or shoved dog may lose control of both hind legs. The dog can be thrown into convulsions at will.
June 27–29. Dog in good condition, quiet, sleeps continually. No active tetany. On the 29th the animal fell down a short flight of steps; went into convulsions and could not be revived.

Protocol, dog 3. Adult, male, white, short-haired, weight 6 kilos. Radiated one hour a day for 9 days. Serum calcium before radiation, 10.1 mgm. Serum Ca, June 18, after nine hours' radiation, 10.8 mgm. Parathyroidectomized June 18. Radiated 3 to 4 hours a day throughout the experiment beginning June 18.

June 19. Normal.
June 20. Normal in a.m. At 2 p.m. suddenly developed tetany. Very spastic. Bled from heart. Serum calcium 7.6 mgm. Symptoms disappeared after radiation.
June 21. Slight tetany.
June 22. Slight tetany in a.m. Developed violent symptoms while being radiated. Serum calcium 5.1 mgm. Symptoms disappeared after radiation. Dog quiet.
June 23. No tetany symptoms in a.m. Mild tetany 8 p.m. Radiated 1 hour and 40 minutes; symptoms disappeared.
June 24. Violent tetany. Radiated 4 hours. Complete recovery after radiation.
June 25. Mild tetany symptoms. Animal in good condition. Serum calcium 6 mgm.
June 26. Dogs shows no tetany but is losing weight.
June 27. Marked tetany. Complete recovery after 3 hours' radiation.
June 28. No tetany; animal not radiated.
June 29. Animal weak; mild tetany; symptoms disappeared after radiation.
June 30. Slight tetany; animal very weak; vomits all food. Given 100 cc. milk by stomach tube. No tetany after radiation.
July 1–5. Mild tetany symptoms. Radiated 3½ hours a day. Dog much emaciated, vomits most of the food given. Dog in a tetanoid condition; sleeps most of the time, but can be thrown into convulsions at any time by shock or rough handling.

July 5–13. Mild tetany which can be temporarily controlled by prolonged radiation. Animal extremely weak and emaciated. Died July 13 of exhaustion. The serum calcium did not rise above 6 mgm. per 100 cc. despite the prolonged radiation.

SUMMARY

1. Exposure of normal dogs to ultra-violet rays for one hour a day for periods ranging from four to twelve days does not appreciably alter the level of the serum calcium. It may even be slightly diminished in some animals.

2. Radiated dogs develop tetany following parathyroid removal as rapidly as normal non-radiated dogs fed the same diet.

3. Radiation of parathyroidectomized dogs greatly prolongs the life of such animals and brings about a striking amelioration of the violent symptoms.

4. Radiated animals may live for twenty-five days after the first appearance of tetany symptoms, but sooner or later die of tetany or exhaustion.

5. The calcium content of the blood serum of such operated dogs is low and prolonged radiation does not raise it to normal.

6. The amelioration of tetany symptoms by ultra-violet rays and prolongation of life of parathyroidecomized dogs is probably due to the effect of such radiation in increasing the absorption and retention of the small amounts of calcium obtained from the food, so that its elimination from the blood and tissues is less rapid than in non-radiated parathyroidectomized dogs.

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ON THE CHEMICAL REGULATION OF RESPIRATION

II. A Quantitative Study of the Accumulation of Lactic Acid in the Isolated Brain During Anaerobic Conditions and the Rôle of Lactic Acid as a Continuous Regulator of Respiration

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There is ample evidence indicating the significance of the hydrogen ion in the chemical control of respiration, but the generally accepted view that the hydrogen ion concentration of arterial blood is the prime factor in this control is open to criticism (1) on the grounds of the numerous instances of lack of parallelism between pulmonary ventilation and acidity of arterial blood. For example, the hyperpnea of anoxemia, in which the blood is more alkaline than normal (2), (3), and the subsequent apnea, which occurs on the administration of oxygen or room air. In this latter case the arterial blood actually increases in acidity due to the oxidation of hemoglobin (4), and to the synchronous increase in alveolar carbon dioxide (5). Apnea occurs despite this increase in acidity of arterial blood. Another instance of lack of correspondence between respiratory activity and hydrogen ion concentration of arterial blood is exemplified in the hyperpnea of hemorrhage associated with increased alkalinity of arterial blood, and the apnea promptly elicited by the injection of gum-saline solution (6). The effect of injection comes on so promptly that it is undoubtedly due to an acceleration of unmodified blood through the center. The gum-saline employed had a pH of approximately 4.0. If the hydrogen ion concentration of arterial blood is the controlling mechanism, respiration should increase upon its arrival at the center. Actually respiration continued to be depressed. These results indicate that the apnea cannot be caused by a lowered acidity of arterial blood, nor can it be explained by a diminished number of hydrogen ions reaching the center per unit time as this is actually increased by injection.

These and other evidences of lack of correspondence between acidity of arterial blood and respiratory activity, however, do not exclude the possibility of the hydrogen ion as a major factor of control but suggest that transport of acid away from the center rather than to the center exerts the dominant effect. This conception involves the acid metabolism of the center itself. If we are justified in reasoning by analogy, assuming that

the respiratory center possesses an acid metabolism similar to that of muscle, there appears to be a mechanism of acid control in accordance with the prevailing facts.

Acid metabolism of tissue bears an important relationship to oxygen supply. This was demonstrated by Fletcher and Hopkins (7), who showed that in resting muscle under anaerobic conditions a continuous production of lactic acid takes place; and that in room air or pure oxygen very little if any lactic acid forms. They likewise showed that lactic acid already formed in fatigued muscle disappears in the presence of oxygen. Later Hill (8) and Meyerhof (9) confirmed their observations in a more quantitative way. The results of Fletcher and Hopkins, of Hill and of Meyerhof appear to be significant in explaining the regulation of respiration (1). Meyerhof found, depending on conditions, that only one-fourth to one-sixth of the lactic acid produced in resting muscle in the absence of oxygen is oxidized in the presence of oxygen, the remainder being converted into some non-acidic precursor; so that only one molecule out of every four, five or six formed is oxidized with the resulting production of three molecules of carbonic acid. Thus two processes occur; a formation and a removal of lactic acid. The equilibrium at any moment between these two reactions, which depends upon the oxygen supply, determine the total acidity of the tissue. The effect of formation and removal of lactic acid on the pH of the tissue may be illustrated by schematic ratios. If the normal pH of tissue is represented by the ratio $\frac{1\ H_2CO_3}{20\ NaHC_3O}$, then any increase in the numerator or decrease in the denominator would increase the acidity and conversely a decrease in the numerator or increase in the denominator would decrease the acidity. It is assumed that the conditions are such that for every five molecules removed one is oxidized under aerobic conditions. Supposing that the oxygen supply is deficient and two molecules of lactic acid fail to be removed. These will combine with $NaHCO_3$ with the formation of two molecules of Na lactate which have only a slight buffering value, and two molecules of H_2CO_3; the normal ratio then becomes $\frac{3\ H_2CO_3}{18\ NaHCO_3}$. The original ratio of $\frac{1}{20}$ is now $\frac{3}{18}$, and the hydrogen ion concentration accordingly increased. If we start now with anaerobic conditions in which an excess of Na lactate is present and with a normal ratio of $\frac{3\ H_2CO_3}{60\ NaHCO_3}$ and still assume that one of every five molecules of lactic acid removed by sufficient oxygen supply is oxidized, an increase in oxygen to the tissue would cause a formation of three molecules of H_2CO_3, and a disappearance of five lactate ions with a liberation of 5 Na ions; that is, four lactate ions return to their precursor state, and one is oxidized—forming three molecules of H_2CO_3. Of the five sodium ions

liberated, three combine with the H_2CO_3 produced, leaving two to react with two molecules of the numerator; so that the normal ratio of $\frac{3\ H_2CO_3}{60\ NaHCO_3+5\ NaL}$ is now $\frac{1\ H_2CO_3}{65\ NaHCO_3}$, and the tissue becomes less acid. Taking the less striking case of one molecule oxidized out of every four removed, the reaction would indicate increased alkalinity, as represented by the ratios $\frac{3}{60}$ and $\frac{2}{64}$.

Bearing these examples in mind, there appears to be an acid mechanism which will explain the hyperpnea of anoxemia, and the depression of respiration on subsequent administration of room air or oxygen.

In this connection there are three questions which arise regarding the rôle of lactic acid as a normal respiratory stimulant. Is lactic acid normally formed in brain tissue; does its concentration there depend upon the balance between aerobic and anaerobic conditions, and lastly, does the amount formed as a result of deficient oxygen supply vary enough in varying degrees of anoxemia to produce changes of sufficient magnitude to account for varying pulmonary ventilation?

To answer the first question there is little direct evidence that lactic acid occurs in brain tissue although there is abundant evidence of its presence in other tissue, especially blood under various conditions (10), (11), (12), (13). Langendorff (14) found a rapid increase in the acidity of the brain when the blood supply was cut off or when the brain was removed. However, he did not estimate lactic acid, and a search of the literature fails to reveal any direct analyses of this tissue. The answer to the second and third questions, of course, involves the first. These questions are of paramount importance in the control of respiration by the acid metabolism of the respiratory center. If it be found that brain tissue acts similarly to muscle tissue, it will be possible to explain more logically the hitherto conflicting facts.

The respiratory center, as pointed out by Haldane, is extremely sensitive to small changes in hydrogen ion concentration,—a rise of 0.2 per cent or 1.5 mm. carbon dioxide tension of alveolar air and arterial blood being sufficient to double the resting pulmonary ventilation. This change in arterial carbon dioxide tension corresponds to a difference in arterial hydrogen ion concentration of 0.012 pH. "The outstanding delicacy of the regulation of blood reaction is thus evident. No existing physical or chemical method of discriminating differences in reaction approaches the physiological reaction" (15). Assuming that a change of 1.5 mm. carbon dioxide tension produces a similar change in hydrogen ion concentration in the respiratory center as in the blood, it should be sensitive to changes in acidity resulting from small changes in its own oxidations.

METHOD AND RESULTS. The method was devised to show, first, the normal content of lactic acid in brain tissue of the normal living animal,

and, second, to learn the rate of accumulation of lactic acid under certain conditions of anoxemia.

For the first series of experiments, rabbits were used. The animal was decapitated by a single blow of a cleaver and the entire head dropped into a vessel of liquid air to insure rapid refrigeration (−190°C.), since it was considered essential to obtain tissue as normal as possible with reference to lactic acid content. The frozen head was split and the tissue removed and weighed. The brain tissue was then crumbled in a chilled mortar and ground to a thin brei with cold sand and 10 to 13 cc. each of cold 10 per cent Na_2WO_3 and $\frac{2}{3}$ normal H_2SO_4. After grinding approximately 40 cc. of water and 5 cc. each of 10 per cent Na_2WO_3 and $\frac{2}{3}$ normal H_2SO_4 were added and the contents transferred to a 100 cc. volumetric flask. The mortar was rinsed with water and the contents of the flask made up to 100 cc.[1] The filtrate which was perfectly clear and colorless was analyzed for lactic acid following the procedure of Clausen (16). Sugar was removed with $CuSO_4$ and $Cu(OH)_2$, as suggested by Van Slyke (17), the tubes were centrifuged, and the clear supernatant fluid was accurately pipetted directly to the oxidation tubes. An equal volume of concentrated H_2SO_4 was added and the oxidation and aeration carried out at 150°C. for two hours. Four determinations were made on each Folin-Wu filtrate, and the results were averaged. The method gave excellent checks. It is undoubtedly true that the results are somewhat high for lactic acid itself. A few direct determinations were made, and it was found that between 75 and 80 per cent of the "lactic acid" in normal brain tissue was lactic acid itself, precipitated as zinc lactate, the balance being bisulfite binding compounds formed by oxidation. It is probable, however, that these compounds are acidic in nature, and exert a similar effect on hydrogen ion changes that lactic acid does. The results given in table 1 show a wide variation in absolute amounts of lactic acid for a given interval of time following decapitation, indicating considerable differences in the lactic acid content of different individuals. For example, the lactic acid content of brain tissue incubated five minutes following removal of the head varies between 69.9 and 122.7 mgm. per 100 grams of brain tissue with an average content of 100.8 mgm. In those experiments in which the head was frozen within two seconds from the time of decapitation, the values range between 65.3 and 96.1 mgm. with an average of 80.8 mgm. It is true that there is an apparent accumulation of lactic acid during the severe anaerobic conditions of incubation, as evidenced by the difference between 80.8 and 100.8 mgm. but a large number of experiments would be required to obtain sufficient data from which to plot a curve showing the rate of formation at any time. To obtain such a

[1] This was the method employed throughout with the exception of the first nine experiments in which Na_2WO_3 and H_2SO_4 were added subsequent to grinding with cold water.

curve with economy of time and animals, the procedure was modified. Small unanesthetized dogs were substituted for rabbits in order to obtain larger amounts of tissue. The animals were beheaded with a specially designed guillotine with a T-shaped blade so arranged that one blow was sufficient to sever the head from the body and split the skull in half. One half of the brain tissue was quickly removed and frozen in liquid air, the other half being incubated at various intervals of time, the whole head being placed in the incubator. The second half was then frozen in the same manner as the first, an accurate record of the time intervening between the fall of the knife and the freezing of the tissue. Differences in lactic acid content of the two halves gave a means of calculating and plotting rate of formation without complications due to variation in different dogs. Sixty

TABLE 1

RABBIT BRAIN	LACTIC ACID IN MGM. PER 100 GRAMS TISSUE	TIME BETWEEN DECAPITATION AND FREEZING OF HEAD	REMARKS
1	42.7	1 minute	
2	69.9	4 minutes	
3	102.6	5 minutes	
4	91.0	5 minutes	Brain not frozen in expt. 7
5	87.1	5 minutes	Na_2WO_3 and H_2SO_4 added to brei after grinding tissue with cold water
6	122.7	5 minutes	
7	105.7	20 minutes	
8	68.6	10 seconds	
9	65.3	½ second	
10	75.4	½ second	
11	83.9	¾ second	
12	83.2	1 second	
13	96.1	2 seconds	

to 80 grams of brain were obtained by this method and two determinations were run on each half. Blood samples were taken in a few dogs at the time of decapitation. The results are given in table 2.

The absolute values for lactic acid content of those brains frozen within ten seconds from the time of decapitation as well as those incubated over various intervals of time are variable just as in the case of the experiments on rabbits; but if one plots the difference in lactic acid content of the two halves on the ordinates against time intervals between freezing on the abscissas, the figures thus obtained fall on a well defined curve (fig. 1 curve *I*). The value of this procedure obviously is better than the former not only because fewer experiments are necessary but because the results from each dog are significant no matter what the initial lactic acid content is.

It will be seen from the curve that the rate of accumulation of lactic acid during the first three minutes of incubation is virtually a linear function of the time, and that approximately 60 mgm. of lactic acid accumulate

during this time or about 1 mgm. every three seconds. The average lactic acid content of those halves frozen within twelve seconds is 75.2 mgm. and the average time is 8.85 seconds, during which time approximately 2.95 mgm. of lactic acid formed; 2.95 mgm. subtracted from 75.2 leaves 72.3 mgm. which is the average initial lactic acid content of brain tissue. The curve may now be plotted using this value of 72.3 mgm. as the basal level (fig. 2, curve *I*).

TABLE 2

DOG BRAIN	TIME REQUIRED TO FREEZE FIRST HALF	LACTIC ACID CONTENT FIRST HALF MGM. PER 100 GRAMS TISSUE	LACTIC ACID CONTENT SECOND HALF MGM. PER 100 GRAMS TISSUE	TIME INTERVAL BETWEEN FREEZING FIRST AND SECOND HALVES	DIFFERENCE IN LACTIC ACID CONTENT OF FIRST AND SECOND HALVES MGM. PER 100 GRAMS TISSUE
1	12 seconds	76.5	87.9	15 seconds	11.4
2	12 seconds	74.2	80.1	15 seconds	5.9
3	30 seconds	74.6	87.9	30 seconds	13.3
4	45 seconds	89.3	98.3	45 seconds	9.0
5	2 seconds	94.2	110.8	1 minute	16.6
6	16 seconds	93.2	119.3	1¼ minutes	26.1
7	7 seconds	79.2	116.9	1½ minutes	37.7
8	60 seconds	73.2	108.9	2 minutes	35.7
9	11 seconds	65.2	115.0	2½ minutes	49.8
10	9½ seconds	79.6	153.5	3 minutes	73.9
11	11 seconds	66.6	131.9	3½ minutes	65.3
12	6 seconds	69.7	150.8	4 minutes	81.1
13	10 seconds	87.1	169.8	4⅚ minutes	82.7
14	5 seconds	83.0	173.9	6 minutes	90.9
15	8 seconds	67.5	148.7	6⅚ minutes	81.2
16	10 seconds	95.8	162.7	6 minutes	66.9
17	8 seconds	75.1	172.0	8 minutes	96.9
18	5 seconds	68.4	170.2	10 minutes	101.8
19	11 seconds	70.0	159.6	12½ minutes	89.6
20	7 seconds	69.9	175.5	15 minutes	105.6
21	10 seconds	65.0	162.5	17½ minutes	97.5
22	9½ seconds	61.2	178.0	20 minutes	116.8
23	20 seconds	66.1	175.7	23 minutes	109.6
24	12 seconds	81.6	193.8	25 minutes	112.2
25	16 seconds	71.5	171.5	27½ minutes	100.0
26	11 seconds	74.0	183.2	30 minutes	109.2

It will be seen that the rate of accumulation of lactic acid for the first five minutes is very rapid, but a maximum is soon reached being due perhaps to a lack of precursor or simply to the mass effect of the lactic acid previously formed. The initial normal concentration is doubled in four minutes, and reaches an approximate value of two and a half times the initial value in ten minutes. Attention may be called to the fact that blood lactic acid of these dogs averaged 23.9 mgm. per 100 cc. showing a

wide difference between brain tissue and blood. It is not the purpose of this paper to explain these observations in a quantitative way.

The question now came up concerning the accumulation of lactic acid in brain tissue under conditions of anoxemia in the living animal. There is ample evidence that anoxemia causes an accumulation of lactic acid in blood (10), (11) and in severe anoxemia in urine (10). Lactic acid in muscle, likewise, fails to be removed when the oxygen supply falls below normal (18). If it does accumulate in brain tissue as a result of anoxemia, what would be the effect on the curve of formation after decapitation? If there is a limited supply of precursor the rate of formation should be slower than the normal rate because the initial value was higher. Experiments were made on dogs after anoxemia of carbon monoxide poisoning.

TABLE 3

DOG BRAIN	TIME REQUIRED TO FREEZE FIRST HALF	LACTIC ACID CONTENT FIRST HALF MGM. PER 100 GRAMS TISSUE	LACTIC ACID CONTENT SECOND HALF MGM. PER 100 GRAMS TISSUE	TIME INTERVAL BETWEEN FREEZING FIRST AND SECOND HALVES	DIFFERENCE IN LATIC ACID CONTENT OF FIRST AND SECOND HALVES MGM. PER 100 GRAMS TISSUE	BLOOD LACTIC ACID MGM. PER 100 CC. BLOOD
1	9 seconds	106.0	179.8	5⅛ minutes	73.8	
2	5 seconds	128.9	234.8	7½ minutes	115.9	79.7
3	9 seconds	88.3	219.6	10 minutes	131.3	39.7
4	14 seconds	138.7	276.0	10 minutes	137.3	
5	7 seconds	132.2	256.3	10 minutes	124.1	84.4
6	12 seconds	101.7	210.5	12½ minutes	108.8	56.0
7	21 seconds	142.0	263.2	12½ minutes	121.2	
8	6 seconds	142.2	270.0	15 minutes	127.7	74.0
9	6½ seconds	156.9	285.0	17½ minutes	128.1	93.3
10	9 seconds	125.6	245.4	20 minutes	119.8	77.3
11	7 seconds	121.8	249.1	22½ minutes	127.3	
12	8 seconds	115.2	245.6	25 minutes	130.4	60.3

To produce a condition of extreme anoxemia, the dog was placed in an air tight metal-lined box of 216 liters capacity, supplied with tubes for introduction of gases and for taking gas samples. The dogs were placed on screens under which were large trays containing 30 per cent sodium hydroxide for the absorption of carbon dioxide. The box was connected directly to a Hutchinson spirometer containing pure oxygen, so that the partial pressure of oxygen within the chamber was maintained at approximately 150 millimeters tension throughout the experiment. Sufficient pure carbon monoxide gas was introduced into the chamber to maintain a constant value of 0.2 per cent (1.5 mm.), making allowance for the absorption of carbon monoxide by the hemoglobin of the blood. A small fan circulated the air within the box to insure rapid absorption of carbon

dioxide and mixing of the gases within the chamber. Small dogs of about the same weight (5 kilos) were used, and they were allowed to remain within the box for one hour, after which they were removed and decapitated as soon as possible; it usually requiring about thirty seconds after opening the lid of the box to place the animal in position under the guillotine. This time is too short to permit of any appreciable desaturation of the blood. Decapitation, incubation of the head, and analyses were carried out exactly as for normal dogs. In the majority of experiments, blood samples were taken at the time of beheading.

The results are tabulated in table 3. The variations are great as was to be expected both for initial values and for those at the end of incubation

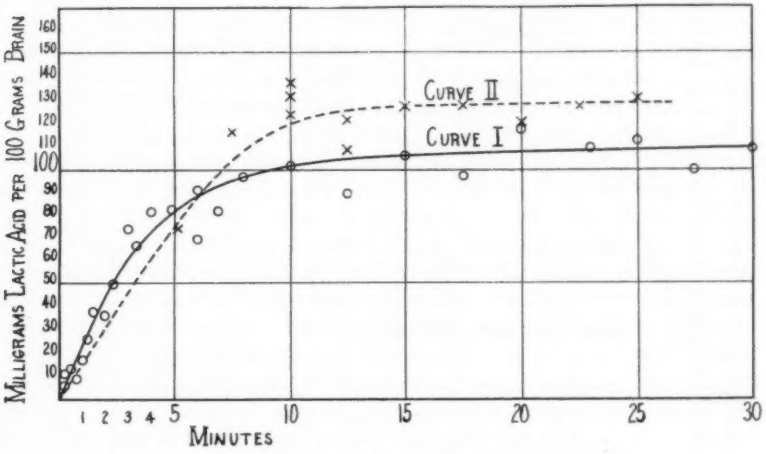

Fig. 1

periods. However, if the differences between lactic acid content of the two halves of brain are plotted against difference in time between freezing, just as in the case of normal dogs, the results fall on a well-defined curve. The curve is quite similar to curve *I* (figs. 1 and 2). (See curve *II*, fig. 1.) The initial value of 121.3 mgm. was obtained in the same manner as for normal dogs, that is, the average lactic acid content (123.4 mgm.) of those halves frozen in an average time of 8.36 seconds was diminished by the amount of lactic acid which presumably formed during this time (2.1 mgm.). The curve was then constructed using 121.3 mgm. as a base line. (See curve *II*, fig. 2.) If the curve of lactic acid formation for normal dogs is plotted on the same graph as the curve for anoxemic dogs, as in figure 1, it will be seen that they are almost identical in form, the curve for anoxemic dogs being somewhat higher, that is, showing a more rapid rate of accumu-

lation of lactic acid during the first ten minutes.[2] Blood lactic acid reached an average value of 75.1 mgm.

Discussion. The results of the foregoing experiments indicate that there is an acid metabolism of the respiratory center similar to that of

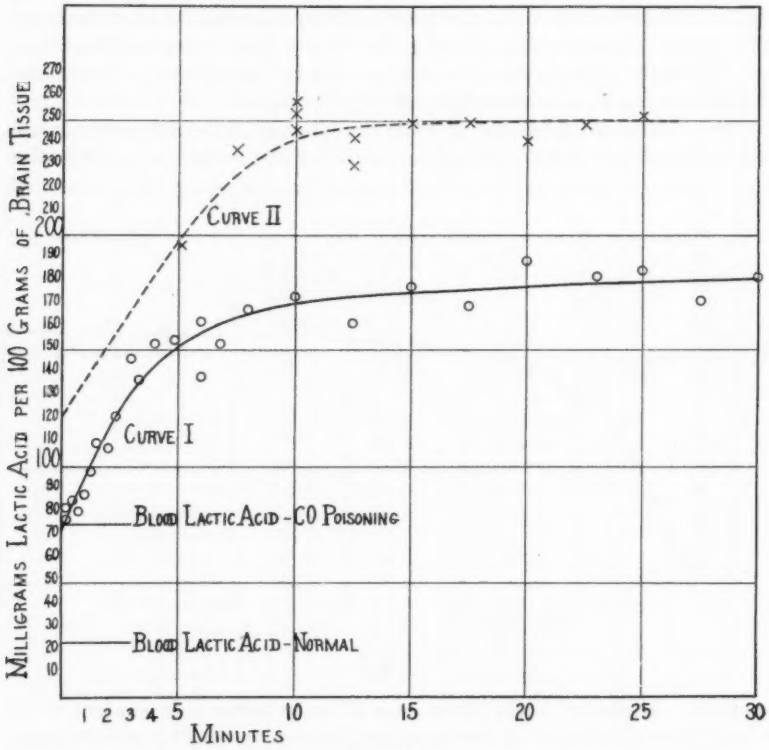

Fig. 2

muscle. Lactic acid accumulates under conditions of oxygen deficiency and in extreme oxygen want, the formation is extremely rapid. Undoubtedly it is true that in the living animal there is a continual formation of

[2] The real values of lactic acid here are also somewhat lower than the curve indicates, direct precipitation experiments showing that seventy to seventy-five per cent of the "lactic acid" can be obtained as the zinc salt. Anoxemia is known to give rise to acidosis and a resulting formation of hydroxy and ketonic acids as well as acetone, all of which are estimated as lactic acid by the method employed. However, these bodies with the exception of acetone are acidic in nature and exert a similar effect on respiratory control that lactic acid does.

lactic acid in the respiratory center, and it seems highly probable that the analogy between brain tissue and muscle tissue may be carried to the removal of lactic acid in the presence of oxygen as well as to its accumulation in the absence of oxygen; the equilibrium point of these reactions depending on the general activity of the tissue and upon the oxygen supply.

Is the formation of lactic acid during oxygen want of sufficient magnitude to account for increased pulmonary ventilation? For some years Haldane and his co-workers (19), (20) held that in the living animal, lactic acid was formed within the respiratory center as a result of anoxemia; the increase in hydrogen ion concentration serving as a respiratory stimulus, or at least lowering the threshold of stimulation to carbon dioxide. If carbon dioxide was blown off by forced breathing, a low oxygen tension had no influence on the center and apnea would result even under these circumstances. Haldane, however, had later given up the idea of lactic acid formation within the center acting as a respiratory stimulus, and adopted the view that oxygen want itself acted on the center either by a direct action or by lowering the threshold of excitability to hydrogen ions. Haldane (15) definitely states that local metabolism within the center is of minor importance, and that excitation of the center depends on chemical stimuli sent to it by its blood supply. There is apparently ample evidence that lactic acid occurs in blood in large amounts during anoxemia. Araki (10) found lactic acid in the organism under any condition that led to oxygen want, and others have arrived at the same conclusion. On the other hand, there are contradictory results. Ryffel (12) could not confirm Araki's work, and found no decided increase in blood lactic acid of man even after breathing an atmosphere containing twelve per cent oxygen for four hours. Violent exercise, however, caused its appearance in blood, and an excretion of considerable amounts in the urine. Barcroft and Ryffel (21), likewise, could demonstrate no increase in the lactic acid content of blood of persons living at high altitudes, although a lowered dissociation constant of oxyhemoglobin indicated the presence of an acid with no change in carbon dioxide tension. Macleod (13) found an increase of lactic acid in blood during anoxemia but stated that this bears no relationship to stimulation of respiration but appears in order to neutralize base liberated by blowing off of carbon dioxide. Objection may be made to many conclusions of the above observations on the grounds that the observations in general were made on blood lactic acid and it appears from our results that the amount of blood lactic acid and lactic acid of the respiratory center may differ considerably. There are two possible explanations of this relation. Lactic acid may accumulate during anoxemia in the tissues before significant increases occur in the blood; or its local occurrence in the respiratory center by stimulating respiration would tend to prevent the general development of anoxemia and therefore the general formation of lactic acid.

The latter view is supported by a comparison of the rates of formation of acid under normal conditions of adequate oxygen supply, and under conditions of anoxemia described in this paper. According to Lusk (22) the basal production of carbon dioxide in man is 0.30 cc. per minute per 100 grams of body weight. During the first three minutes of incubation the brain tissue lactic acid content increased by 60 mgm., which is equivalent to approximately 14.6 cc. of carbon dioxide or 4.87 cc. of carbon dioxide per minute per 100 grams of brain tissue. If we assume that the metabolism of the brain is the same as that of the body as a whole, one can see here that the acid production during anoxemia is approximately sixteen times greater than that under normal conditions. Some allowance should be made for the higher metabolic rate of the dog, but if we assume that the basal metabolism of brain tissue is twice or three times that of the body as a whole, there is still a safe margin of increased acid production during anoxemia over that of the normal basic conditions.

What effect does this accumulation of acid have upon the hydrogen ion concentration of the brain? Obviously, without knowing the buffering properties of brain tissue one cannot calculate the change in acidity, but assuming that brain tissue has the same buffering capacity as blood, the experiments of Hartree and Hill (23) give an approximate answer to the question. They added 100 mgm. of lactic acid to 100 cc. of defibrinated sheep's blood kept at a constant tension of 41 mm. of carbon dioxide. The C_H increased 2.2 times its original value or a change in pH from 7.24 to 6.97. To be sure, conditions of severe anoxemia are seldom attained in the living animal; yet bearing in mind the extreme sensitivity of the center to changes in CO_2 tension, as demonstrated by Haldane, the increased production of acid even during relatively low degrees of anoxemia must be felt by the center.

It seems justifiable to accept the fact that an acid metabolism occurs in the respiratory center similar to that of muscle, and that the accumulation of lactic acid during oxygen want is of sufficient magnitude even during conditions of moderate anoxemia to account for changes in pulmonary ventilation accompanying these conditions. To test out this hypothesis, one must explain the lack of correspondence between pulmonary ventilation and the hydrogen ion concentration of the arterial blood. One typical example may suffice.

Administration of air low in oxygen leads to hyperpnea due to a failure to oxidize and remove lactic acid which is continually being produced within the respiratory center. Lactic acid accumulates, and by virtue of its hydrogen ion concentration the center is stimulated to activity. Arterial blood may become more alkaline than normal because of blowing off carbon dioxide by the increased pulmonary ventilation, and because of its greater content of reduced hemoglobin. Here we have an alkaline blood

and an acid center during the hyperpnea of oxygen want. Administration of room air or of oxygen leads to a prompt increase in the acidity of arterial blood because of the oxidation of hemoglobin, and the subsequent liberation of carbon dioxide. The mechanism within the center is likewise prompt. Increased oxygen supply facilitates the rapid removal of the excess lactic acid, the hydrogen ion concentration diminishes, and apnea occurs. The center becomes alkaline during the time the arterial blood becomes acid. Thus the respiratory center and arterial blood may vary in hydrogen ion concentration in opposite directions, and the lack of correspondence between pulmonary ventilation and hydrogen ion concentration of arterial blood is explained. Obviously, the conditions of the above example are extreme, and under such conditions lactic acid might function as a respiratory stimulant. The question arises, does it function under usual conditions? The mere fact that lactic acid is present in the brain of the living animal, indicates that there is a continual anaerobic metabolism. The curve indicates that this metabolism may change at any moment, that even a slight reduction in oxygen supply leads to accumulation of lactic acid. If such is the case, lactic acid metabolism within the center does serve as a respiratory stimulant. In order that such a mechanism might function the center would have to be in a continual state of finely adjusted equilibrium between oxygen need and adequate oxygen supply. There is some more or less indirect evidence that this is true. Verzar (24) found that only a very small reduction in the volume-flow of blood to a tissue resulted in reduced oxygen consumption. Krogh (25) says that normally the oxygen tension of tissue approaches zero, and that during rest most of the capillaries are closed. In our experiments the shape of the curve suggests that accumulation of lactic acid begins on the instant that the blood supply is shut off.[3] The reverse is undoubtedly true, that disappearance of lactic acid begins on the instant that adequate oxygen supply reaches the center. Unquestionably lactic acid metabolism changes immediately with changes in oxidation, and presumably with any small changes in oxygen supply.

[3] One other question which came up was the explanation of the shape of curve *II*. The total amount of lactic acid formed in brain tissue by incubation after anoxemia of carbon monoxide poisoning is slightly greater than that formed in incubated brain tissue of normal animals. There is a possibility that anoxemia causes a mobilization of lactic acid precursor. Araki (10) and others agree that anoxemia, no matter what its cause, produces an increase of glucose in blood, especially in well-fed animals. Macleod and Hoover (26) state that injection of glucose into the blood causes no change in lactic acid content. Meyerhof (9) finds, however, that such substances as glucose, hexose diphosphoric acid, or glycogen when present with minced muscle do not hasten the formation of lactic acid, but the total amount formed is greater.

SUMMARY AND CONCLUSIONS

The theory of the continuous regulation of respiration by the lactic acid content of the respiratory center is put to the test.

This test involves the demonstration of lactic acid in the brain of the healthy animal under natural conditions, and an increased content during lack of oxygen.

The heads of resting unanesthetized dogs were severed and split with one quick stroke of a T-shaped guillotine. One-half of each brain was promptly frozen in liquid air, and the other halves after varying intervals of incubation at body temperature. Lactic acid dererminations established the basal level and the rate of accumulation during lack of oxygen.

The basal level was 72.3 mgm. per 100 grams of brain tissue.

The curve of lactic acid accumulation showed an abrupt rise during the first four minutes which gradually changed to the horizontal in the twenty minutes following.

During the period of rapid accumulation, lactic acid accumulated at the rate of 20 mgm. per 100 grams of tissue per minute.

This rate of acid formation is approximately sixteen times the basal production of carbon dioxide of man.

Assuming a cerebral basal metabolic rate three times the basal rate of man, the acid production during lack of oxygen is still five times as great as during oxygen plenty.

Granting brain tissue a buffering capacity equal to the blood, and granting the respiratory center a moderate sensitivity to changes in hydrogen ion concentration, the hyperpnea of lack of oxygen and depression of respiration with oxygen plenty are explained.

Carbon monoxide anoxemia in the living animal increased the lactic acid content of the brain and blood from 73.2 mgm. and 23.9 mgm. per 100 grams of tissue respectively to 121.3 mgm. and 75.1 mgm. respectively.

These changes offer ample explanation of the hyperpnea of carbon monoxide poisoning on the acid basis.

The theory that carbon dioxide is the "normal" regulator of respiration, and that lack of oxygen is the "abnormal" stimulus operating only under conditions of stress appears untenable, for the equilibrium between anaerobic and aerobic production of acid demands differences in total acid simultaneous with the smallest fluctuations in oxygen supply.

The curve of lactic acid gives no indication of a latent period intervening between oxygen lack and lactic acid accumulation.

The data support the view that lactic acid is an important regulator of respiration. This is tantamount to saying that oxygen regulates respiration indirectly by determining the amount and kind of acid formed within the respiratory center.

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FACTORS INFLUENCING THE RATE OF OXYGEN CONSUMPTION IN UNICELLULAR ORGANISMS[1]

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Anesthetics and starvation are known to decrease metabolism in the higher animals, whereas low temperatures, food and thyroxin increase it. The object of this investigation is to determine the effect of anesthetics, temperature, starvation, food and thyroxin on the rate of oxygen consumption in unicellular organisms.

Paramecium caudatum and Colpoda (Sp.?) were the unicellular organisms used. These were grown at ordinary room temperature (20°C.) on infusions made of dried pond lily leaves and tap water. Five hundredths cubic centimeter of the organisms was used for each determination. The 0.05 cc. measure was made by sealing off one end of a 10 cm. piece of a 1.0 cc. glass pipette graduated to hundredths of a cubic centimeter. After collecting, centrifugalizing and washing the organisms with aerated tap water, they were introduced into the measure. The measure was then placed in a small centrifuge making approximately 1200 revolutions per minute and centrifugalized for one minute. The debris which collects on the surface and the excess of organisms were drawn off by means of a fine-pointed pipette. The 0.05 cc. of the organisms left in the measure was then transferred to dropping bottles of 40 cc. capacity filled with aerated tap water. The oxygen content of the water in the bottles containing the organisms, as well as that of the control bottles, was determined at the end of one hour according to the Rideal-Stewart (1901) modification of the Winkler method. The liberated iodine was titrated with N/400 sodium thiosulphate. It was found necessary to invert the bottles occasionally to prevent the organisms from settling to the bottom of the bottles.

The experiments to be described now were carried out to determine the effect of anesthetics on the oxygen consumption of the unicellular organisms, Paramecium caudatum and Colpoda (Sp.?). The anesthetics used were ether, chloroform, nitrous oxide and ethylene. The ether and chloroform were administered by bubbling air through the anesthetic

[1] Data taken from the thesis submitted to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the Ph.D. degree.

and then through the water containing the organisms at the rate of a bubble every few seconds. The nitrous oxide and ethylene were both administered with oxygen by bubbling these gases through the water containing the organisms.

After collecting and washing a large quantity of paramecia with aerated tap water, they were divided into 5 portions of approximately 100 cc. each. The normal oxygen consumption of 0.05 cc. of the organisms taken from each of these portions was determined. The remaining organisms were then subjected to the effect of the various anesthetics, and the results of typical experiments are shown in figure 1, *anesthetics*. By examining the curve marked ether, it may be seen that this anesthetic decreased the oxygen consumption of paramecia 4 per cent in 30 minutes, 19 per cent in 1 hour, and 27 per cent in 2 hours. The curve marked chloroform shows that this anesthetic decreased the oxygen consumption of paramecia 10 per cent in 30 minutes, and 24 per cent in 1 hour. By examining the curves marked nitrous oxide and ethylene, it may be seen that these anesthetics had little or no effect on the oxygen consumption of paramecia. It should be stated in this connection that while a deep anesthesia was produced with ether and chloroform, it was impossible to produce any anesthesia with nitrous oxide or ethylene. It was also found that chloroform was more effective in producing anesthesia than was ether. At the end of 1 hour of chloroform anesthesia and 2 hours of ether anesthesia, the organisms were removed from the liquid through which the anesthetic had been passed and transferred to fresh liquid. It may be seen that 4 hours later, the oxygen consumption of the organisms that had been subjected to the chloroform for 1 hour had decreased still further, and at the end of 24 hours, upon examination of the liquid with a microscope, it was found that most of the organisms were dead. On the other hand, the oxygen consumption of the organisms that had been subjected to ether for 2 hours had returned to within 12 per cent of the original normal at the end of 24 hours. It should be stated that if the chloroform was administered for only 30 minutes and the organisms then transferred to fresh liquid, that at the end of 24 hours the oxygen consumption of these organisms had returned practically to the original normal, just as was the case with the organisms subjected to ether. These experiments would seem to indicate that chloroform is a more powerful anesthetic and more toxic to the unicellular organism, paramecium, than is ether.

It was found that if a mixture of colpodae and paramecia was subjected to ether or chloroform, the colpodae were anesthetized more quickly than were the paramecia, as indicated by the fact that when the colpodae had practically ceased to move, the paramecia were still swimming about very vigorously. It was also found that when a mixture of these organ-

isms was subjected to nitrous oxide or ethylene, no anesthesia was produced in paramecia, whereas a slight anesthesia was produced in colpodae. A number of experiments similar to the preceding were carried out and it was invariably found that chloroform and ether anesthetized the organisms and decreased the oxygen consumption, whereas nitrous oxide and ethylene produced little or no anesthesia and had little or no effect on oxygen consumption.

In figure 1, *temperature*, is shown the effect of different temperatures on the oxygen consumption of paramecia. A large quantity of the organisms was collected and divided into 5 portions of approximately 100 cc. each. After determining the oxygen consumption of the normal organisms, the different portions were placed in baths at 0°C., 5°C., 10°C., 15°C., and 32°C. respectively. It may be seen in figure 1 that the oxygen consumption of the organisms kept at 0°C. for 5 hours had been decreased 58 per cent; of those kept at 5°C., 50 per cent; of those kept at 10°C., 34 per cent; of those at 15°C., 30 per cent; and that the oxygen consumption of those kept at 32°C. for 1 hour had been increased 35 per cent. It should be mentioned in this connection that the temperature of the liquid containing the normal organisms was 20°C. By comparing these results it may be seen that the effect of the temperatures lower than the normal was to decrease the oxygen consumption of paramecia, whereas the temperature higher than the normal increased it. At the end of the experiments, the organisms were removed from the different baths and allowed to stand at the normal temperature (20°C.) until the end of 24 hours, when determinations were again made of the oxygen consumption. It may be seen in the figure that at this time the oxygen consumption of the organisms that had been kept at the lower temperatures had increased well nigh to the original normal, whereas the oxygen consumption of the organisms that had been kept at 32°C. had decreased below that of the normal organisms. This decrease was probably due to the injurious effect of the higher temperature. In all, 5 experiments similar to the preceding were carried out with fairly uniform results.

A fall in outside temperature is known to increase oxygen consumption in warm-blooded and decrease it in cold-blooded animals. The preceding experiments show that the oxygen consumption of paramecia is decreased with a fall in temperature and increased with a rise. Hence, these organisms react to a change in the temperature of their environment as do cold-blooded animals.

The following experiments show the effect of starvation on the oxygen consumption of paramecia. A large quantity of the organisms was collected, centrifugalized, washed, and the oxygen consumption determined in the usual manner. The organisms were then transferred to approximately 300 cc. of aerated tap water free from food material. At certain

intervals during the period of starvation, the oxygen consumption of the organisms was determined. The results of the determinations are shown in figure 1, *starvation*. It may be seen that at the end of 18 hours of starvation, the oxygen consumption of the paramecia had decreased 15 per cent; at the end of 24 hours, 23 per cent; at the end of 48 hours, 27 per cent; and at the end of 72 hours it had decreased 29 per cent. By comparing these various periods, it is apparent that the greatest decrease

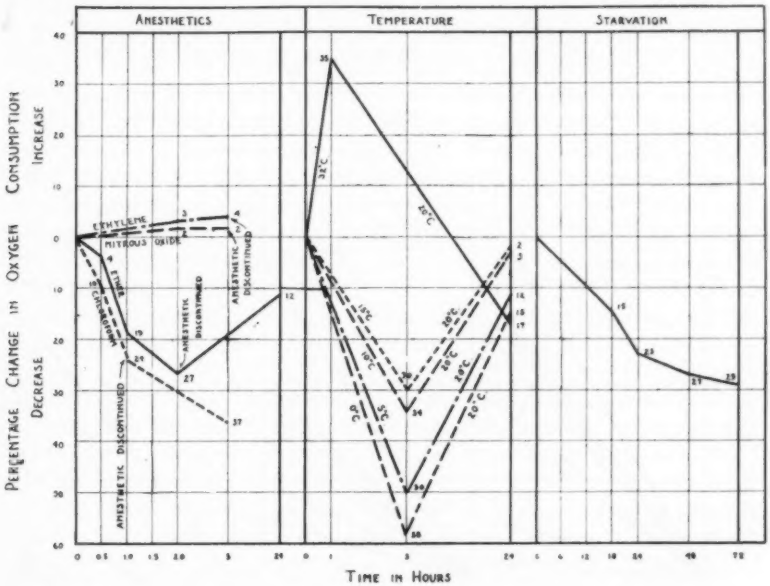

Fig. 1. *Anesthetics*. Curves showing that ether and chloroform decrease oxygen consumption in paramecia, whereas nitrous oxide and ethylene have no effect.

Temperature. Curves showing the decrease in oxygen consumption of paramecia brought about by lowering the temperature, and the increase by raising it.

Starvation. Curve showing the decrease in oxygen consumption of paramecia brought about by starvation.

in oxygen consumption occurred during the first 24 hours of starvation, and that thereafter the decrease was slight. Examination under the microscope showed a progressive decrease in the size of the organisms and increase in the transparency of the cell protoplasm during the period of starvation. The results of the preceding experiments are representative of a number of experiments carried out on the effect of starvation on the oxygen consumption of paramecia.

The paramecia used in the study of the effect of food were starved for

24 hours previous to beginning the experiments to reduce their metabolism to a basal level. The food materials studied were peptone, aminoids, glycocoll, phenyl alanine, caprine, iso-leucine, glutamic acid, tyrosine, cystine, succinic acid, soluble starch, dextrin, maltose, lactose, sucrose, dextrose and galactose.

Approximately 0.2 cc. of paramecia, previously centrifugalized, washed, and on basal metabolism, was added to 100 cc. of a 0.1 per cent neutral solution of each of the following: peptone, aminoids, succinic, and the various amino acids enumerated. These different solutions were neutralized by the addition of sodium bicarbonate. Determinations of the

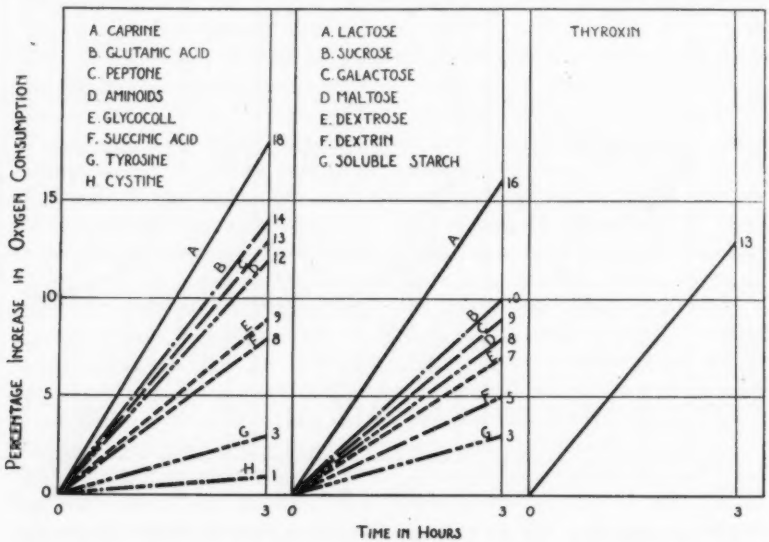

Fig. 2. Curves showing the increase in oxygen consumption of paramecia brought about by the substances indicated.

oxygen consumption of these organisms were made at the beginning of the experiment and after 3 hours. It may be seen in figure 2, *caprine*, etc., that peptone increased the oxygen consumption of the paramecia 13 per cent in three hours; that the aminoids increased it 12 per cent; glycocoll, 9 per cent; caprine, 18 per cent; glutamic acid, 14 per cent; and succinic acid, 8 per cent. Although not shown on the chart, it was found that phenyl alanine increased the oxygen consumption 9 per cent and iso-leucine, 13 per cent. Tyrosine and cystine were found to have little or no effect on the oxygen consumption of paramecia, which was probably due to the fact that these substances are practically insoluble in water.

By comparing these various percentage increases, it may be seen that caprine produced the greatest and succinic acid the least increase. The fact that caprine produced the greatest increase in oxygen consumption is of no particular significance, since in the several experiments carried out, this result was not invariably obtained. However, it should be stated that all of the amino acids produced a great increase in the oxygen consumption of the organisms, sometimes one producing the greatest increase and again another.

In figure 2, *lactose*, etc., is shown the effect of soluble starch, dextrin, maltose, sucrose, lactose, dextrose and galactose on the oxygen consumption of paramecia. Approximately 0.2 cc. of the organisms whose metabolism had been reduced to a basal level was added to 100 cc. of a 0.4 per cent solution of these various substances. It may be seen that soluble starch increased oxygen consumption in paramecia 3 per cent in 3 hours; dextrin increased it 5 per cent; maltose, 8 per cent; lactose, 16 per cent; sucrose, 10 per cent; dextrose, 7 per cent; galactose, 9 per cent. These curves were constructed from data obtained from the average results of 5 experiments.

From the preceding it may be seen that the amino acids increased the oxygen consumption of paramecia more than did the carbohydrates, although only one-fourth as much of the amino acids was used as of the carbohydrates. It is known that the amino acids stimulate metabolism in the higher animals to a greater extent than do the carbohydrates. These observations on paramecia show that this also holds true for these unicellular organisms.

The following experiments were carried out to determine the effect of thyroxin on the oxygen consumption of paramecia. Approximately 0.2 cc. of the organisms which were on basal metabolism was introduced into 100 cc. of aerated water in which a 2-grain thyroid tablet had been dissolved. Determinations of the oxygen consumption were made at the beginning and after the experiments had been going for 3 hours. The curve under *thyroxin* in figure 2 was constructed from data obtained from the average results of 5 experiments. It may be seen that thyroxin produced an average increase in the oxygen consumption of paramecia of 13 per cent. The greatest increase in oxygen brought about by thyroxin in these 5 experiments was 16 per cent and the least increase 8 per cent. The administration of thyroxin to the higher animals greatly increases the respiratory metabolism. The results obtained in these experiments show that thyroxin has a similar effect on the oxygen consumption of the unicellular organism, paramecium.

Discussion. A great many theories have been advanced in an attempt to explain the mode of action of anesthetics in producing anesthesia. John Snow (1858) in his work on *Chloroform and Other Anesthetics* was

the first to suggest that narcotics may produce narcosis by limiting or interfering with the normal oxidative processes. Hans Meyer (1899) and E. Overton (1901) independently advanced theories of anesthesia which were essentially the same. These investigators claimed that anesthetics produce their characteristic effect by combining with the vitally important cell lipoids, in this way changing their relationship to the rest of the cell constituents and resulting in a decreased cellular activity or anesthesia. Verworn (1912) attributes the action of anesthetics to an asphyxiation of the cells. He claims that these substances prevent the transmission of oxygen from the surrounding medium to the oxidizable material. Paul Bert (1890) and Arloing (1890) found that during chloroform anesthesia there was a decrease in the amount of oxygen absorbed and carbon dioxide exhaled. Furthermore, they showed that the longer the anesthesia was continued, the greater was the decrease in respiratory metabolism. In the present investigation it was found that chloroform and ether, which produced a deep anesthesia in the unicellular organisms, also greatly decreased oxygen consumption, whereas nitrous oxide and ethylene, which produced little or no anesthesia, had little or no effect on oxygen consumption.

The influence of temperature on the rate of metabolism of warm-blooded, cold-blooded and hibernating animals has been repeatedly determined. As a result of the work of Lavoisier (1777) and of a great number of investigators since his time, it is now known that a fall in outside temperature produces an increase in the rate of metabolism of warm-blooded animals and a decrease in the rate of metabolism of cold-blooded animals. In warm-blooded animals, body temperature is maintained at a constant level for a long period of time in spite of wide variations in outside temperature due to the mechanisms of chemical and physical regulation. Cold-blooded animals have no heat-regulating mechanism or else a very poorly developed one, and hence the body temperature of these animals varies directly as the outside temperature. As a result of a long series of experiments on the lower marine invertebrates, Vernon (1896) reports that there appears to be a constant relationship between temperature and the respiratory exchange of cold-blooded animals. He finds that the percentage increase or decrease in the rate of metabolism varies in different animals, but as a rule, the less differentiated the animal the greater is the effect produced. Barratt (1905) determined the carbon dioxide production in paramecia at various temperatures and found that more than twice as much carbon dioxide was produced at a temperature of 27° to 30°C. than at 15°C. The increase in oxygen consumption observed in paramecia in the present investigation over this range of temperature agrees very well with the increase in carbon dioxide production reported by Barratt.

It is a well-established fact that when an animal is deprived of food,

metabolism decreases until it reaches a certain low level at which it remains fairly constant. In the present investigation it was found that starvation decreased the oxygen consumption of the unicellular organism, paramecium, just as is known to be the case in the higher animals.

Lavoisier (1780) was the first to observe that the ingestion of food increased the rate of oxidation in the body. Rubner (1902) showed that the ingestion of protein brought about a greater increase in the rate of metabolism than did the fats, and that these in turn had a greater stimulating effect than did the carbohydrates. That this stimulating effect of the food materials was due to a direct action on the body cells and not through a stimulation of the nervous system, was demonstrated by Tangl (1911) who observed an increase in the metabolism of curarized dogs after the ingestion of large amounts of protein. A number of theories have been advanced in an attempt to explain the mode of action of the food materials in increasing metabolism. Voit (1881) claimed that the presence of large amounts of food substances increased the power of the cells to metabolize. Lusk (1915) suggests that the increase in metabolism may be due to the stimulating action of metabolic products, such as glycollic and lactic acids, while Grafe (1915) attributes the stimulating action of protein to the amino groups. That the ingestion of certain amino acids produces an increase in metabolism was first demonstrated by Lusk (1912). He reports that glycocoll and alanine exert a powerful stimulating effect, leucine and tyrosin have but a slight effect, whereas no effect is obtained with glutamic or succinic acids. Grafe, on the other hand, states that glutamic acid also increases metabolism. In the present investigation it was found that the amino acids, glycocoll, phenyl alanine, caprine, iso-leucine and glutamic acid, produced an increase in the rate of oxygen consumption of paramecia. Tyrosine and cystine had little or no effect, as might be expected from the fact that these amino acids are practically insoluble in water. Succinic acid, which according to Lusk may be a product of the metabolism of glutamic acid, was also observed to increase oxygen consumption in paramecia, although it would seem to be less effective than were the amino acids. Increases in oxygen consumption of paramecia were obtained with all of the carbohydrates used. These substances were less effective than were the amino acids, in keeping with the fact that they do not exert as great a stimulating effect on the metabolism of the higher animals.

Thyroxin, the active principle of the secretion of the thyroid gland, is known to exert a powerful influence on general metabolism. Andersson and Bergman (1898) attribute the increased metabolism following thyroid ingestion and in exophthalmic goiter to an increase in muscular activity or in muscle tone, due to a great increase in the irritability of the central nervous system. In the work reported in this paper it was found that thyroxin produced an increase in the rate of oxygen consumption of the unicellular organism, paramecium, just as it does in the higher animals.

SUMMARY

Powerful anesthetics, such as chloroform and ether, produced a deep anesthesia and a decrease in oxygen consumption in the unicellular organisms, paramecium and colpoda. Colpodae were found to be much more easily anesthetized with these anesthetics than were the paramecia. It was impossible to anesthetize or to decrease the oxygen consumption of paramecia with the weaker anesthetics, such as nitrous oxide and ethylene, while a slight degree of anesthesia and a small decrease in oxygen consumption were produced in the more easily anesthetized organisms, colpodae.

A fall in the temperature of the environment of paramecia produced a decrease in the oxygen consumption of these organisms, and a rise in temperature brought about an increase in oxygen consumption.

Starvation was found to decrease oxygen consumption in paramecia.

Glycocoll, phenyl alanine, iso-leucine, caprine, glutamic acid, succinic acids, aminoids and peptone increased the rate of oxygen consumption of paramecia, while tyrosine and cystine, which are practically insoluble in water, had little or no effect.

All of the carbohydrates used, dextrose, galactose, sucrose, lactose, maltose, dextrin and soluble starch, increased the rate of oxygen consumption. However, they were less effective in this respect than were the amino acids.

Thyroxin was found to produce an increase in the rate of oxygen consumption of paramecia, resembling in this respect its effect on the metabolism of the higher animals.

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EFFECTS PRODUCED BY EXTRACTS OF PARATHYROID GLANDS ON NORMAL AND PARATHYROIDECTOMIZED DOGS

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Since our previous report (Fisher and Larson, 1925) on the effects of extracts of the parathyroid glands we have accumulated considerable data on the chemical changes in the blood of normal and thyro-parathyroidectomized dogs following the subcutaneous injections of various extracts of ox parathyroid glands. The effectiveness of such extracts, in relieving parathyroid tetany and the temporary prevention of further attacks, have been carefully noted. Moussu (1898) was able to temporarily relieve tetania parathyreopriva by subcutaneous and intravenous injections of water or glycerine extracts of horse parathyroids. MacCallum (1906) had some success in controlling parathyroid tetany by the intravenous or intraperitoneal injections of large quantities of emulsions of either fresh or dried parathyroid glands. Berkeley and Beebe (1909) prepared the nucleoprotein from ox parathyroids which was effective in the relief of parathyroid tetany. Recently Collip et al. (1925a, b, c) have reported the chemical changes in the blood of normal and parathyroidectomized dogs produced by extracts of ox parathyroids which were effective in the relief of parathyroid tetany. Hanson (1924), (1925) has reported the preparation of an active extract of parathyroids which will increase the calcium content of the blood as well as relieve parathyroid tetany. No attempt will be made to make a detailed review of the extensive literature on the parathyroid glands. For a review of the literature the following may be consulted: Boothby (1921) and Simpson (1922).

Procedure. A number of dogs were thyro-parathyroidectomized by removing the thyroid capsule and the contained thyroid and parathyroid glands. The blood vessels were doubly ligated about 1 inch from the glands. The diet consisted of hashed meat for the purpose of hastening and increasing the severity of the tetany following parathyroidectomy. The extracts were always administered by subcutaneous injections. The blood for the chemical analysis was drawn from the leg veins. The variations in calcium, phosphates, non-protein nitrogen and blood sugar were

followed for periods preceding the injection of extracts and for periods as long as 21 days after the first injection.

The calcium determinations were made by the modified Tisdall method as suggested by Clark and Collip (1925). For the blood sugar determinations the Folin-Wu method has been used and the inorganic phosphorus has been determined by the Tisdall method (1922). At first the Folin-Wu method for non-protein nitrogen was used but later the Koch-McMeekin method (1924) was found to be more satisfactory, not only as regards the time factor, but for the prevention of the turbidity due to the silica formed during digestion.

Methods of preparing the extracts. Extract A. Some of the ox glands received from the stockyards were ground in a meat chopper and heated at 70°C. for 5 minutes with 100 cc. of normal HCl per ounce of glands. After cooling, the congealed fat was removed mechanically. The remaining material was ground for 4 hours in a ball mill. Upon neutralizing with NaOH a large amount of protein separated. After adding acid till the point of maximum precipitation of protein was reached, the precipitate was removed by filtration. The precipitate was redissolved in alkali and reprecipitated with acid. After evaporation under a 40°C. air blast to the desired concentration, the extract was preserved with 0.1 per cent tricresol.

Extract B. Three volumes of 95 per cent alcohol were added to one volume of extract A. The slight precipitate was removed by filtration and the filtrate then concentrated and preserved as before.

Extract C. Extract A was half saturated with ammonium sulphate, filtered and the ammonium sulphate removed by dialysis in a collodion bag against tap water.

Extract D. To the ground glands, four times their weight of 3 per cent acetic acid in 95 per cent alcohol was added. After standing for 12 hours the residue was removed by filtration and again extracted for 12 hours with two portions of 3 per cent acetic acid in 60 per cent alcohol. These acid alcohol extracts were evaporated under the air blast. The remaining solid was dissolved in water and preserved.

Extract X. The insulin method as used by one of us (Fisher 1923–1924) was applied to the ox parathyroids. Previous to the extraction the glands were freed from fat, ground, and placed in a ball mill with the acid alcohol mixture and ground for 20 hours.

Extract M. Extract M was made by the following process: 100 grams of the glands were ground with sand in a mortar and extracted for 16 hours with a mixture of 120 cc. of 95 per cent alcohol, 30 cc. of water, and 40 cc. of concentrated hydrochloric acid. After straining through cheese cloth, the residue was reëxtracted for 6 hours with the same amount of 60 per cent alcohol. The solutions were combined, neutralized, filtered, and the filtrates then concentrated.

Extract L. Extract L was prepared by extracting 100 grams of the ground glands with 200 cc. of 80 per cent alcohol for 16 hours. After filtration this extract was concentrated. Both the M and L extracts were brown in color.

Protocol, dog 10. Male mongrel. Weight, 12 kilos.

March 25. Thyro-parathyroidectomy
March 26. 1 pound meat
March 27. 1 pound meat
March 28. 2:00 p.m. Severe tetany. 3½ cc. Ex. C
2:30 p.m. Tetany
March 29. Condition excellent. Ate 1 pound meat greedily
8:45 p.m. Severe tetany. 10 cc. Ex. M
10:15 p.m. Much improved
March 30. Ate 1 pound meat eagerly
March 31. a.m. 1 pound meat
9:00 p.m. Signs of tetany. 8 cc. Ex. M
April 1. Appetite good
April 2. Slightly depressed
April 3. Excellent condition. Ate meat voraciously
April 4. Ate 2 pounds meat
April 5 to June 1. Placed in a large pen and kept on a meat diet

Effects of extracts on the chemical constituents of the blood

TIME	EXTRACT GIVEN	NON-PROTEIN NITROGEN PER 100 CC. OF BLOOD	SUGAR PER 100 CC. OF BLOOD	CALCIUM PER 100 CC. OF SERUM	INORGANIC PHOSPHORUS PER 100 CC. OF SERUM	REMARKS
Series I. Dog 10. April 28 to May 8						
hours		*mgm.*	*mgm.*	*mgm.*	*mgm.*	
0	6 cc. Ex. A*	32.2	75.0	6.9	7.89	
1	6 cc. Ex. A*					
20	6 cc. Ex. A*	40.2	76.8	10.91	5.18	
23	6 cc. Ex. A*					
24	6 cc. Ex. A*					
25	6 cc. Ex. A*					
29¼	6 cc. Ex. A*	52.37	72.5	8.61	4.37	
31¾	6 cc. Ex. A*	45.7	78.2	9.15	4.16	
43½		33.07	96.1	11.05	4.62	Atonia, depression, ataxia
49¾		81.7	120.5	11.03	5.66	Vomited
74¼		45.9	67.8	9.68	6.41	Ate vomitus
121½		73.02	75.3	7.57	7.98	
237¾		30.2	87.8	5.8	6.15	

TIME	EXTRACT GIVEN	NON-PROTEIN NITROGEN PER 100 CC. OF BLOOD	SUGAR PER 100 CC. OF BLOOD	CALCIUM PER 100 CC. OF SERUM	INORGANIC PHOSPHORUS PER 100 CC. OF SERUM	REMARKS
Dog. 10. Series II. May 15–18						
hours		*mgm.*	*mgm.*	*mgm.*	*mgm.*	
0	6 cc. Ex. B*	39.37	86.2	5.65	7.98	No tetany
2½	6 cc. Ex. B*					
3½	6 cc. Ex. B*					
4¾	6 cc. Ex. B*	37.87	76.95	4.94	4.72	No tetany
22	6 cc. Ex. B*			6.05	6.37	No tetany
23	6 cc. Ex. B*					
24	6 cc. Ex. B*					
27¾	6 cc. Ex. B*	35.6	75.2	6.53	4.75	No tetany
47	6 cc. Ex. B*	35.6	75.6	7.09	7.24	No tetany
53¼		44.0	83.8	7.09	6.60	No tetany
81¼		32.1	82.0	8.36	5.98	No tetany

* One cubic centimeter = 1 gland.

June 2 Returned to a cage. Eyes infected. Eyes washed with boric acid solution

June 3. Eyes much improved

June 4. Found dead. Death due to acute dilatation of the stomach. Mass of hair the size of 2 large fists impacted against the pyloric sphincter

Protocol, dog 12. Male poodle. Weight, 7 kilos.

May 5. Thyro-parathyroidectomized

May 6. 12:15 p.m. 1 pound meat
5:00 p.m. 10 cc. Ex A. Calcium of blood serum 7.6 mgm. per 100 cc. Inorganic phosphorus of blood serum 6.37 mgm. per 100 cc.
8:00 p.m. Depressed. Had vomited
9:00 p.m. No tetany. Drinks
10:30 p.m. No tetany but somewhat depressed

May 7. 12:15 p.m. 1 pound meat
5:00 p.m. Tetany 7 cc. Ex. A

May 8. 8:00 p.m. 5 cc. Ex. A. Blood non-protein nitrogen—54.2 mgm. per 100 cc. Blood sugar—95.5 mgm. per 100 cc. Blood serum calcium—8.28 mgm. per 100 cc. Blood serum inorganic phosphorus—6.48 mgm. per 100 cc.

May 9. 12:15 p.m. Ate 1 pound meat voraciously, 6 p.m. 5 cc. Ex. A

May 10. 12:15 p.m. Ate 1 pound meat voraciously, 6 p.m. 5 cc. Ex. A

May 11. 12:15 p.m. Ate 1 pound meat voraciously, 6 p.m. 5 cc. Ex. A

May 12. 12:15 p.m. Ate 1 pound meat voraciously, 6 p.m. 5 cc. Ex. A

May 13. Ate ½ pound meat. Later vomited

May 14. 5 cc. Ex. A

May 15. No injection

May 16. 5 cc. Ex. A

May 17. Ate meat

May 18. Ate meat. Condition excellent

May 19. Ate meat. Condition excellent

May 20. Depressed. Refuses food

May 21. 10:00 p.m. Tetany 7 cc. Ex. A

May 22. Very active. Ate meat ravenously
May 23. Ate a little meat
May 24. Very depressed. Refuses food
May 25. Very depressed. Refuses food.
May 26. Very depressed. Refuses food
May 27. 6:30 p.m. Tetany. 10 cc. Ex. B
May 28. a.m. Tetany
4:00 p.m. Found dead. Tetany

Effect of parathyroid extract on the chemical constituents of the blood of normal dogs

TIME	EXTRACTS INJECTED	NON-PROTEIN NITROGEN PER 100 CC. BLOOD	SUGAR PER 100 CC. BLOOD	CALCIUM PER 100 CC. SERUM	INORGANIC PHOSPHORUS PER 100 CC. SERUM	REMARK
Dog 25. Male airedale. Weight, 15 kilos						
hours		*mgm.*	*mgm.*	*mgm.*	*mgm.*	
0	3 cc. Ex. A	38.2	87.0	12.4	5.36	
6	3 cc. Ex. A	31.9	89.6	13.39	3.83	
18	3 cc. Ex. A	31.94	93.0	17.87	4.42	
25½	3 cc. Ex. A	37.65	84.1	19.72	5.21	Depression, atonia, ataxia
45	3 cc. Ex. A	109.8	100.0	20.1	7.25	Blood concentrated
78½		111.02	85.0	15.37	8.79	
98		104.7	95.8	14.04	8.07	
122½		139.5	79.4	11.74	12.17	
170½		105.2	73.6	12.77	9.19	No albumen in urine
194½		72.5	73.4	10.37	5.38	No albumen in urine
218½		104.1	65.84	9.71	6.18	Creatinine in blood 1.65 mgm. per 100 cc.
342		77.4	90.4	10.26	6.53	
501¾		39.38	80.4	10.34	3.86	
Dog 26. Series I. Male shepherd. Weight, 13 kilos						
0	6 cc. Ex. A	41.1	90.7	10.35	3.53	
2	6 cc. Ex. A					
20	6 cc. Ex. A	50.3	69.8	13.47	3.95	
23	6 cc. Ex. A					
24	6 cc. Ex. A					
25	6 cc. Ex. A					
29	6 cc. Ex. A	50.3	84.8	11.42	4.38	
31½	6 cc. Ex. A	49.7	73.95	10.92	5.49	
43¼		93.8	110.2	14.64	6.39	Depressed, blood concentrated
49½		104.7	96.2	12.74		
74		212.9	78.9	11.92	9.2	
103½		177.5	79.8	10.59		
121¾		92.0	73.8	9.8	5.68	
237¾		72.0	80.7	9.87	3.84	
397½		42.7	58.1	9.17	4.95	

TIME	EXTRACTS INJECTED	NON-PROTEIN NITROGEN PER 100 CC. BLOOD	SUGAR PER 100 C.C. BLOOD	CALCIUM PER 100 CC. SERUM	INORGANIC PHOSPHORUS PER 100 CC. SERUM	REMARKS
		Dog 26. Series II				
hours		*mgm.*	*mgm.*	*mgm.*	*mgm.*	
0	10 cc. Ex. C	42.7	58.1	9.17	4.95	
2¾	10 cc. Ex. C					
3¾	10 cc. Ex. C					
4¾	10 cc. Ex. C	62.7	75.5	8.12	3.85	
22	10 cc. Ex. C			9.25	4.74	
23¼	10 cc. Ex. C					
24¼	10 cc. Ex. C					
28¾	10 cc. Ex. C	56.8	84.2	7.1	5.38	
47¼	10 cc. Ex. C	32.2	93.2	6.99	5.50	
53			93.9	8.42	5.63	
69¼		44.9	87.2	7.04	7.97	
		Dog 27. Young male mongrel. Weight, 27 kilos				
0	6 cc. Ex. A	38.8	113.7	9.33	4.1	
1	10 cc. Ex. A					
2	10 cc. Ex. A					
3	10 cc. Ex. A					
4¾	10 cc. Ex. A					
5¾	10 cc. Ex. A					
6¾	10 cc. Ex. A	36.77	117.7		4.87	
19¼		47.4	130.0	17.38	3.65	Depressed, atonia
26¼		56.4	110.4	13.1	5.75	
50¾		76.4	108.0	9.34	5.26	
98½		55.9	82.7	9.57	6.64	
241		48.94				
		Dog 28. Male. Weight, 13 kilos				
0	6 cc. Ex. C	48.9	103.5	9.33	6.85	
1	6 cc. Ex. C					
2	6 cc. Ex. C					
3	6 cc. Ex. C					
4½	6 cc. Ex. C					
5½	6 cc. Ex. C					
6¾	6 cc. Ex. C	42.3	93.2	12.37	7.05	
19¼		30.56		12.1	5.96	
26¼		61.1	77.6	10.1	8.93	
50¾		36.05	111.6	9.2	6.46	
98½		50.6	105.8	9.3	7.46	

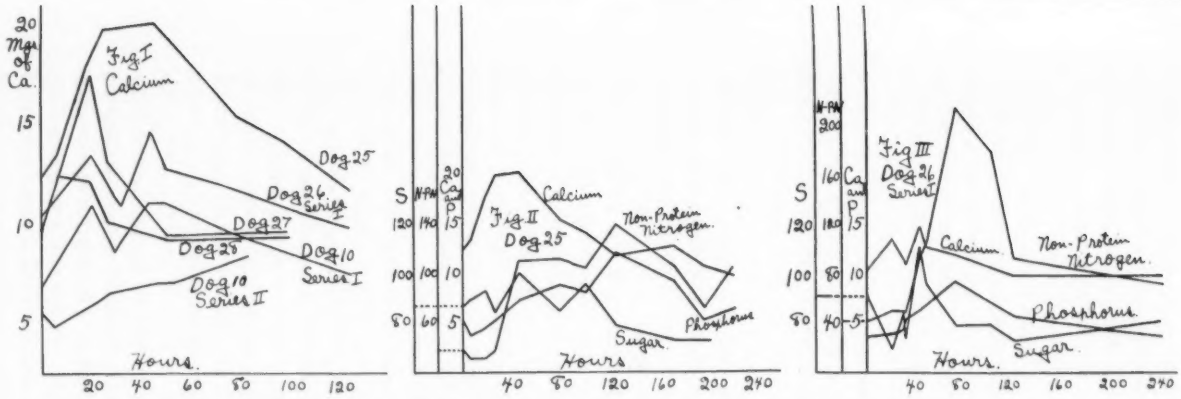

Fig. 1. The variations of the calcium in the blood serum. Injections of parthyroid extract as follows:

Dog 25. Normal. Male. Weight 15 kilos. Three cubic centimeters Extract A. One cubic centimeter = 1 gland at 6, 18, 25½ and 45 hours after the initial injection.

Dog 26. Normal. Male. Weight 13 kilos. Six cubic centimeters Extract A. One cubic centimeter = 1 gland at 2, 20, 23, 24, 25, 29 and 31½ hours after the initial injection.

Dog 27. Normal. Male. Weight 27 kilos. Six cubic centimeters Extract A. One cubic centimeter = 2 glands for the initial injection and then 10 cc. Extract A. One cubic centimeter = 2 glands at 1, 2, 3, 4¾, 5¾ and 6¾ hours.

Dog 28. Normal. Male. Weight 13 kilos. Three cubic centimeters Extract C. One cubic centimeter = 2 glands at 1, 2, 3, 4½, 5½ and 6¾ hours after the initial injection.

Dog 10. Male. Parathyroidectomized. Weight, 12 kilos.

Series 1. Six cubic centimeters Extract A. One cubic centimeter = 1 gland at 1, 20, 23, 24, 25, 29¼ and 31¾ hours after the initial injection.

Series 11. Six cubic centimeters Extract B. One cubic centimeter = 1 gland at 2½, 3½, 4¾, 22, 23, 24, 27¾ and 47 hours after the initial injection.

Fig. 2. Relative variations of blood constituents.

Dog 25. Normal. Male. Weight, 15 kilos. Three cubic centimeters Extract A. One cubic centimeter = 1 gland at 6, 18, 25½ and 45 hours after the initial injection.

Fig. 3. Relative variations of blood constituents.

Dog 26. Normal. Male. Weight, 13 kilos. Six cubic centimeters Extract A. One cubic centimeter = 1 gland at 2, 20, 23, 24, 25, 29 and 31½ hours after the initial injection.

Discussion. The above protocols are two typical ones that have been selected at random from a series of eighteen animals. Several of the dogs were given but one or two injections of extracts of parathyroid glands to relieve the early attacks of tetany and then carefully observed to learn the developments resulting from the omission of the extracts. In every case except dog 9, which will be referred to later, death due to tetany followed when the extracts were omitted soon after parathyroidectomy. When the extracts were administered continually and tetany prevented for a period of two or three weeks, it was found that tetany did not develop for a long period of time and the animals remained in excellent condition while being kept on a meat diet.

In case of one dog tetany following parathyroidecomty was prevented by daily injections of extracts for the first five days. On the second day following the last injection latent tetany, as indicated by tremors, was completely relieved by a second injection. No symptoms of tetany were apparent for eight days following this injection. At this time mild tetany was present at intermittent periods for three days. During this time the animal ate considerable amounts of meat and appeared in good condition. On the third day of this tetany manifestation, the eleventh day since the last injection of extract, very severe tetany ensued. The attack was relieved by an injection of extract. On the second day following this injection symptoms of tetany became apparent and were relieved by another injection of the extract. The condition of the animal was good for three days more when another injection of extract was given to relieve the tremors. The dog has now been in excellent condition for the last 106 days without any injections while kept on a meat diet. The blood serum calcium determinations have been as follows: 5.32, 8.83 and 7.05 mgm. per 100 cc.

Dog 8 showed tetany on the third day following parathyroidectomy and the condition required almost daily injections of extract for a period of 12 days to control the tetany. This was followed by a period of 76 days during which tetany was absent. At this time blood samples were drawn and it was found that the calcium of the serum was considerably below normal. Though the dog had a good appetite he became quite thin. The dog died on the 93rd day following thyro-parathyroidectomy of severe colitis. The dog had been kept on a meat diet during the 93 days which no doubt contributed to the severity of the colitis and aggravation of the state of latent tetany.

Dog 10 behaved similarly to dog 8, tetany developing on the third day following the removal of the glands. Several attacks of tetany during the next 11 days were controlled by the extracts. No tetany was evident during the succeeding 38 days following the omission of the extracts. At this time the animal was used to determine the chemical changes produced in the blood by injection of extracts. A low value for calcium was found

at the beginning which was rapidly increased for a short period of time following the injections. This dog also lost weight in spite of a good appetite. The animal showed a decided susceptibility to eye infections. The increased susceptibility of such animals has been previously reported by Carlson (1912–13a). This dog died 69 days after being thyro-parathyroidectomized as a result of acute dilatation of the stomach secondary to the impaction of a mass of hair the size of two large fists against the pyloric end of the stomach. The dog did not show tetany for 63 days preceding death, although the calcium of the blood serum was as low as 4.16 mgm. per 100 cc.

The case of dog 12 differed somewhat from the other three which have been considered. It was necessary to inject extract almost every day for a period of 16 days in order to relieve tetany. The behavior of this dog was peculiar, being depressed at times, and then again in excellent condition. This dog died in tetany on the 23rd day following parathyroidectomy. Although the tetany had been controlled by parathyroid extract for several days, there was no apparent immunity against further attacks which was evidenced by death on the second day following the last injection of extract.

On the second day following thyro-parathyroidectomy, dog 18 was seized with severe tetany which was relieved with extract. Tetany was again manifested in 24 hours which was relieved by extract. Following this injection, though the animal was kept on a meat diet, no signs of tetany were apparent for 3 days when the animal had a mild seizure. Extract was injected which promptly relieved the attack. The animal has not shown an attack since and is in excellent condition at present, 71 days after the operation. This phenomenon has been observed in a number of cases, namely, that the attacks become more mild and that more time intervenes between the attacks. This is a confirmation of the results of Luckhardt et al. (1922b, 1923) on the control of tetany. The calcium determinations of the blood have been as follows: 25 days after the operation 10.9 mgm. per 100 cc. and 37 days after the operation 11.38 mgm. per 100 cc. serum.

There were 12 control dogs which were thyro-parathyroidectomized and closely observed to determine the rapidity of the onset of tetany and the outcome of the tetany without treatment when the dog was kept on a meat diet. Death resulted in every case in 18 to 96 hours. Some of the animals died during the first attack. The animals which developed tetany soon after the operation had the most severe attacks. Though the attacks occurred as early as 18 to 48 hours, this was not an indication that the tetanic seizures would have been more difficult to control by means of the extract. That is, the severest attacks of tetany readily yielded to treatment as evident from the cases of dogs 8, 10, 15 and 18. In these animals

the attacks became more mild and fewer in number as the experiment progressed. In the cases of 2 dogs that lived 93 and 69 days, there was a marked loss of weight but no signs of tetany for at least 2 months preceding death in the case of dog 10 and only slight tremors in the case of dog 8. Dog 8 had a few tremors and low calcium in the blood serum just before death but these at most seemed to be merely contributing factors and not the real cause of death.

The relation of the parathyroids to the maintenance of the calcium level in the blood serum may be indirect and not entirely necessary since in some cases the calcium level has been maintained near the normal level for several months following the removal of the thryo-parathyroid apparatus. A blood serum sample from dog 15, 61 days after the operation and 15 days after receiving the last injection of extract, contained 8.83 mgm. per 100 cc. In the case of dog 18, 23 days after thyro-parathyroidectomy and 19 days after the administration of extract, the calcium was 10.96 mgm. per 100 cc. of blood serum. The calcium of the blood serum of dog 9 was 8.12, 7.56 and 10.91 mgm. per 100 cc. 3 months after thyro-parathyroidectomy.

Luckhardt et al. (1922a, b, 1923) have reported that thyro-parathyroidectomized dogs after the control of tetany by the oral administration of calcium lactate for several months or by the intravenous injections of Ringer's solution for 40 days, require no further treatment except during the oestrous cycle, pregnancy, lactation or after eating putrid meat. Dragstedt et al. (1923a, b, c) controlled tetany by a diet of lactose, milk and white bread. In our cases we controlled the acute attacks of tetany without such treatment. We purposely kept the animals on a meat diet to aggravate tetany so as to thoroughly test the value of the extracts.

In our experiments, after the alleviation of the acute attacks of tetany, some of the animals became quite emaciated and died. This emaciated condition has been noticed by Thompson, Leighton and Swarts (1909) who ligated the parathyroid artery which produces according to these investigators a chronic cachexia and a stuporous condition ending in death, rather than death in violent tetany. Previously Thompson and Leighton (1908) found upon ligation of the parathyroids that there was a gradual and progressive loss of weight and a diminished resistance to infection.

Following the removal of the parathyroid glands tetany usually develops when the blood serum calcium falls below 7 mgm. per 100 cc. This has been previously reported by Collip (1925a) and others. This has been observed by us many times but we feel that this is only true for about two weeks following the operation. Considering the case of dog 10 the following blood serum calcium values were obtained: 6.9, 5.8, 5.65, 4.94, 6.05 and 6.53 mgm. per 100 cc. With dog 8 calcium values of 4.15 and 3.94 mgm. per 100 cc. of blood serum were obtained. One of the calcium analyses of dog 15 was 5.34 mgm. None of these dogs had shown any signs of tetany for a con-

siderable time previous to the taking of the samples. These are below the value stated by Collip and others which will cause tetany parathyreopriva. Following the removal of the parathyroids there is a decided decrease in the calcium of the blood serum. From our results it seems that this lowered calcium content of the blood serum bears a relation to tetany following parathyroidectomy until adjustment on the part of the body is established. That is, tetany usually occurs when the calcium level falls below 7 mgm. per 100 cc. blood serum soon after thyro-parathyroidectomy. After the acute attacks of tetany have been controlled the calcium level of the blood may fall as low as 4 mgm. without signs of tetany.

Dog 9 has a very unique history. Thyro-parathyroidectomy was performed and the animal kept on a milk and meat diet for 5 days. The animal was then given meat only and signs of tetany were apparent 2 days later and became gradually worse for the succeeding 5 days when extract was administered. After the symptoms of vomiting and marked depression were relieved the animal appeared normal. Eight weeks after the operation the dog gave birth to 5 healthy pups. The dog had been kept on a meat diet since the operation with the exception of the first 5 days. No signs of tetany were apparent at either parturition or during the period of lactation. This animal probably has accessory parathyroid tissue because Carlson (1912–13b) found that pregnancy intensified parathyroid tetany and Luckhardt and Rosenbloom (1922b) had great difficulty in controlling the parathyroid tetany in pregnant and lactating dogs. At present 164 days have elapsed since the operation and the mother and pups are in good condition. The calcium determinations of the blood serum have been as follows: 8.12, 7.48, 10.91 and 11.71 mgm. per 100 cc.

Fourteen dogs were thyro-parathyroidectomized and observed to determine the suddenness of the onset of tetany as well as to test the potency of the extracts. We were also interested in determining the effectiveness of the extract in relieving tetany as well as delaying future attacks. Some of these dogs died in tetany during our absence. Pneumonia was the cause of the death of four dogs. This speaks for a lowered resistance to respiratory infection which has been previously noted by Carlson (1912–13a).

The HCl acid extracts which we have prepared are similar to those of Collip (1925a) and Hanson (1924), (1925). Most of our findings are in close agreement with the results of these investigators. The active substance is evidently quite stable because in the preparation of extract A the glands are kept at 70°C. for 5 minutes with normal HCl. After keeping extract A for 3 months or more in an icebox it was still potent. This does not seem to be in accord with the findings of Berkeley and Beebe (1909) who state that boiling or heating for ½ hour at 80°C. com-

pletely destroys the activity and that it deteriorates rapidly when kept in a refrigerator. They consider the active principle to be an enzyme. Our results as well as those of Collip et al. (1925a, b, c) and Hanson (1924), (1925) make this view untenable. The various methods of preparation which we have used all produced extracts which would alleviate tetany but most of our work was done with extract A which we consider to be the most effective in relieving tetany as well as producing profound changes in the chemical constituents of the blood. In the alleviation of tetany with our extracts our results are different than those of Collip et al. (1925a, b, c) because we have not found it necessary to give daily or almost daily injections for months to prevent tetany in the majority of cases.

The extracts which we have prepared from the ox parathyroids have a decided physiological action, when injected subcutaneously, either into normal or parathyroidectomized dogs, to increase the level of the calcium in the blood serum as well as relieve parathyroid tetany. This decided increase of calcium in the blood serum or hypercalcemia is most effectively produced by a series of successive doses of the extract. This pyramiding of the blood serum calcium is due to the cumulative action of the successive injections. After hourly injections for 6 or 7 hours the calcium reaches its maximum value in 8 to 20 hours depending on the size of the dose and the potency of the extract. The time for the return to normal depends upon the height attained. In case of dog 25 (fig. 1) where the calcium reached a value of 20 mgm., it was 70 hours before the calcium returned to the original level. In the case of 3 other dogs the return to normal level was rapid. The symptoms of overdosage of extract or hypercalcemia as described by Collip (1925a), can be readily induced. The viscosity of the blood is markedly increased. Besides the atonia, profound depression and anorexia mentioned by Collip as symptoms of overdosage of parathyroid extract, ataxia was evident as well. Vomiting was noticed in one case only. From our results it does not seem that all these symptoms of overdosage of parathyroid extract are due to the hypercalcemia.

The rise of the non-protein nitrogen in the blood seems to parallel the calcium of the blood serum, but the latent period for the rise to the maximum and the decrease to normal, is much longer. The maximum rise of non-protein nitrogen to 212.9 mgm. observed in dog 26 (fig. 3) did not take place till the 74th hour while the highest calcium value was at the 43rd hour. The return to normal for the calcium required about 60 hours as against 323 hours for the non-protein nitrogen. The results with dog 25 were quite similar. The increase of the non-protein nitrogen in dog 26 was greater than Collip et al. (1925b) observed in some terminal cases.

The concentration of inorganic phosphorus is increased in the blood serum after the injections of the parathyroid extracts, but the increase occurs later than the increase of the other constituents.

The increase in blood sugar (figs. 2 and 3) produced by the extracts at the time of hypercalcemia can be accounted for by the increased concentration of the blood.

CONCLUSIONS

1. Comparatively pure extracts of the active principles of ox parathyroids for the relief of tetania parathyreopriva have been prepared by various methods.

2. Extract A especially increases the amounts of calcium and inorganic phosphorus in the blood serum and the non-protein nitrogen of the blood of normal and parathyroidectomized dogs.

3. When such extracts are kept at a low temperature and preserved with 0.1 per cent tricresol there is little loss of potency for periods of 3 months.

4. Parathyroidectomized dogs can be kept for a period of at least 7 months on a meat diet providing the acute attacks of tetany are controlled with extracts of parathyroid glands.

5. The acute attacks of tetany usually becomes less severe and disappear entirely in from 1 to 3 weeks when treated with parathyroid extracts, after which further injections are not necessary, at least during a period of 7 months.

6. A low calcium value following the control of the acute attacks of tetania parathyreopriva does not indicate impending tetany.

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THE ALTERED METABOLISM OF NORMAL ANIMALS UNDER INSULIN TREATMENT

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The experimental data as to the precise effect of insulin on the metabolism of carbohydrate are still very conflicting. The very complete review in Ergebnisse der Physiologie by Grevenstuk and Laqueur (1925) exhibits a wide diversity of opinion, particularly on the significance of the respiratory quotients. Two serious criticisms may be urged regarding many of the studies reported. One is the inadequate number of observations, and the other is the unsuitable method adopted for measuring the change in respiratory metabolism. Satisfactory evidence of the total alteration in the character of the foodstuffs oxidized cannot be obtained by frequent, interrupted observations which continue for only a few minutes. There must be continuous measurement of the respiratory gases and there should be simultaneous collection of urine for determination of the protein metabolism. So far as we have been able to discover none of the investigators has attempted to evaluate his results in the light of the oxygen made available by the substitution of more carbohydrate in combustion for fat or protein.

The observations reported below were begun nearly two years ago and grew out of the early experience in this laboratory with insulin in depancreatized animals. We had been struck with the diminished oxygen absorption where a dynamic action of the excessive carbohydrate already in circulation might have been expected when insulin made it available. For example, in the second perfusion paper by Murlin, Clough, Gibbs and Stone (1923) there was in one instance (p. 355, dog 25, August 18) coincident with a fall in blood sugar a rise in R.Q. from 0.73 to 0.83 caused by a 30 per cent reduction in the oxygen absorption and a 20 per cent reduction in CO_2 elimination. Again (p. 358, dog 26, August 22) coincident with an abrupt fall in blood sugar, a rise in R.Q. from 0.70 to 0.85 was caused by a 41.5 per cent reduction in oxygen absorption and a 29.5 per cent reduction in CO_2 elimination.

In the first paper on Aqueous Extracts of Pancreas by Murlin, Clough,

Gibbs and Stokes (1923) one dog (p. 290, dog 10) given a concentrated extract together with 300 cc. of 0.05 N NaOH and 20 grams glucose by stomach tube showed a change in R.Q. from 0.708 to 0.757, 0.796, 0.923 and 1.03 in successive periods caused by a continuously declining absorption of oxygen but a much more constant elimination of CO_2. With the next dog however, the rise in R.Q. was caused by a relatively greater elimination of CO_2.

On referring back to the earlier investigation of Murlin and Kramer (1916) it was found that several experiments which were interpreted at the time as giving evidence of combustion, exhibited the same character of change. Thus, to give a single instance, in one experiment (p. 529, dog 53, June 27) the R.Q. changed as the result of giving pancreas extract with 0.05 N NaOH and glucose by stomach, from 0.70 to 0.72 and 0.83. The first period showed a considerable increase in oxygen absorption and a slightly greater increase in elimination of CO_2. In the next period when the R.Q. was highest the CO_2 elimination remained at the level already attained but the oxygen absorption decreased sharply, producing a net change from the fore period of -6 per cent. In certain other experiments there was an apparent dynamic action, but, even when this occurred, the increase in R.Q. was due to a smaller effect on the oxygen than on the CO_2. None of the pancreas preparations used in the work cited were purified.

The present work on normal rabbits was undertaken primarily for the purpose of comparing the effects of different insulin preparations. It was our belief that the depression of metabolism, when it occurred, was due to certain impurities and it was so stated in the preliminary communication by the present writers (1924). Some confirmation of the view that the effect on the respiratory metabolism may be modified by impurities has been found but a complete report will be reserved for a later paper. We have now to report that the early rise in oxygen absorption followed by a depression such as was seen in the experiments with depancreatized dogs in 1916 and later seems to be characteristic of the action of insulin in sub-convulsive doses on normal rabbits also. It will be found that this change is perfectly consistent with the view that insulin causes an increased combustion of glucose in these animals and with the observation of a diminished supply of glycogen in the liver as found by Dudley and Marian (1923), Babkin (1923), Macleod (1923), von Brugsch (1924), Cori (1924) and others.

METHOD. To establish a fall in oxygen absorption, particularly if it be a very moderate one, convulsions or even the premonitory stage of convulsions must be avoided if possible and one must have evidence that the animal is equally quiet as regards voluntary motions in the fore period as in the after period. Both objects were accomplished in this

series by means of a graphic registration[1] of all movements on the part of the animal. In only one instance (G1 on May 26, 1924, see table 1) were the preconvulsive tremors seen in the graphic record.

Standard animal. The rabbits received the same care and were on the same basal diet of alfalfa hay, oats and potato or carrot as the rabbits used in testing the potency of the insulin products. (Clough, Allen, Root, 1923.) Food was removed from the rabbit to be used at 5:00 p.m. and since the basal period was obtained on the following morning, the rabbit was in a post-absorptive state having had nothing but water for approximately eighteen hours. At least three days were allowed to elapse between two experiments on the same rabbit. Large rabbits were used in preference to small ones. They varied in weight from five to nine pounds. The larger rabbits giving a larger respiratory exchange in a period naturally gave a smaller percentage error on weighing of the absorbers. A rabbit whose O_2 consumption would be at least one liter in the period was used whenever possible.

Apparatus. Respiratory exchange was obtained in two different machines, both of the Benedict closed-circuit type, and essentially alike. With the first, oxygen consumption was measured by a small meter, barometric pressure and temperature corrections being made on the O_2 readings. The second machine was the one used and described by Murlin (1915) in work on metabolism of infants and as later modified (Murlin, Conklin and Marsh, 1925). In this the O_2 was obtained by difference in weight of the very small O_2 cylinder. All but the first few experiments were carried out with this apparatus.

For greater accuracy a residual system was introduced into the second machine. By means of this an analysis of the air, for H_2O and CO_2 content, was obtained at the beginning and end of each period and correction was made for any change. The system served also as an excellent check on the efficiency of the CO_2 and H_2O absorbers.

Barometric and temperature corrections were made on the chamber volume as with the first machine.

Alcohol checks were run whenever the absorbers were changed, or when the basal R.Q. differed from the average basal, or when an unusual quotient was obtained. From the grams of alcohol burned by the small flame, which replaced the rabbit in the box, the theoretical CO_2 and O_2 were calculated and the weights compared with the actual values ob-

[1] The recording mechanism consisted of a wooden rack sensitively hinged at the back of the cage and suspended on a wire spring of suitable distensibility at the front. Upon the rack rested the wire cage in which the animal was contained inside the respiration chamber. A thin-walled pneumograph attached to the front of the rack below and to the support for the spring above communicated by a rubber tubing with a tambour outside. The mechanism was so sensitive as to record at times the respirations of the animal.

tained. Any variation of more than one or two hundredths from the theoretical 0.667 was investigated and the difficulty corrected before more experiments were carried out.

Two successful O_2 checks were made on the machine. In an O_2 check the change in box volume due to a sharp drop in temperature should exactly account for the extra O_2 admitted.

Procedure. All periods were approximately 45 minutes long. An average preliminary of 15 minutes was run before period I in each case to insure an equilibrium of conditions. In some of the early experiments two basal periods were run but the R.Q.'s were so consistent that in later work the second basal was omitted. At the end of the basal period the animal was catheterized, a blood sample drawn and insulin injected subcutaneously. A preliminary period followed by two insulin periods was then obtained. In five of the experiments, after the two-hour blood and urine were taken, the rabbit was again returned to the box and a third and fourth insulin period run. An examination of the four-hour blood and urine was made in such experiments.

The time relations may be more clearly seen in the following general scheme.

9:00 a.m.	Animal catheterized and placed in box
9:00 to 9:15	Preliminary period
9:15 to 10:00	Basal period
10:00 to 10:15	Catheterization, blood drawn, insulin given
10:15 to 10:30	Preliminary
10:30 to 11:15	First insulin period
11:15 to 12:00	Second insulin period
12:00 to 12:15 p.m.	Catheterization, blood (2 hours)
12:15 to 12:30	Preliminary period
12:30 to 1:15	Third insulin period
1:15 to 2:00	Fourth insulin period
2:00	Catheterization, blood (4 hours)

The insulin given was prepared in this laboratory and the dosage was proportional to the weight of the rabbit at the time of injection. The "Rochester Rabbit Unit," the amount necessary to give a 0.070 gram drop in blood sugar in a 2-kilo standard rabbit, was used. The theoretical amount sufficient to give a 0.070 gram drop was subcutaneously injected.

Several types of insulin preparations were given as can be seen from the tables.

Most of these preparations represent different steps in the method described from this laboratory by Allen, Piper, Kimball and Murlin (1923). The essential steps are:

1. Extraction of the macerated pancreas in four volumes of 5th normal hydrochloric acid heated to 75°C.

2. Neutralization of the excess acid to pH of approximately 4.1 and filtration.

3. Reëxtraction of the precipitate formed by neutralization in hydrochloric acid of pH 2.0.

4. Precipitation of insulin from the combined filtrates with sodium chloride, 35 grams to 100 cc. of fluid.

5. Extraction of the salt precipitate with 80 per cent alcohol, distillation in vacuo, and reëxtraction with alcohol several times to remove salt.

6. Precipitation of insulin from the 80 per cent alcohol extract with three volumes of amyl alcohol.

In the table, P9 is a salt precipitate. P8 is a mixture of salt precipitate and amyl alcohol precipitate with 0.1 per cent HCl added. E6PS is a first filtrate without further purification. E5 and 6 is a mixture of the first two filtrates. E11S(2) and E(29)30S are amyl alcohol precipitates. P49 is a perfusate, without further purification, prepared by the method described by Murlin, Clough, Gibbs and Stone (1923).

Basal, two-hour and four-hour blood samples were analysed for sugar by the Folin and Wu method. Benedict's qualitative test for sugar and the regular Kjeldahl determination for nitrogen were made in the urine.

Rectal temperature was recorded in one series of five rabbits (see page 128), and in many of the metabolism experiments.

Experimental results. Several experiments were discarded because the animal was not sufficiently quiet. All those which could be accepted as adequately controlled are shown in table 1. In two of these experiments the blood sugar change was not obtained and in one the insulin preparation proved to be impotent. The rabbit (L1, February 25) which received an insulin preparation having no potency may be taken as a control. The blood sugar in this instance fell only nine milligrams per cent, which is within the limit of error of the Folin-Wu method and there was no material change in the R.Q.

In nine of the seventeen remaining experiments which show a distinct fall in the blood sugar, there is a fall of quotient the first period after insulin. In four the figures for the first period were of no value due to a leak in one train of absorbers. In three there was an increase over the basal in the first insulin quotient. In one of these the rise was due to increase in CO_2 only. In the other two both an increase in CO_2 and a decrease in O_2 contributed to the result. In two there was no change of R.Q.

In all of the experiments there was a rise in R.Q. the second period after insulin. The O_2 consumption in ten of the seventeen treated with a certainly potent preparation decreased below the basal level as the quotient rose. In six the O_2 value rose above the basal in this period and in one it remained the same.

TABLE 1

Summary of individual experiments

DATE	ANIMAL NUMBER	WEIGHT	PREPARATION	PERIOD	O_2 PER HOUR	CO_2 PER HOUR	CALORIES PER KILO PER HOUR	R.Q.	BLOOD SUGAR	URINARY NITROGEN PER HOUR	REMARKS
1923		*kilos*			*liters*	*liters*			*grams*	*grams*	
11/28	F9	2.5	E_6PS	Basal I	1.86	1.33	3.51	0.73	Nor 0.127		
				Basal II	1.83	1.35	3.47	0.74	2 hr. 0.085		
				Insulin I	1.67	1.21	3.17	0.72	Dr. 0.042		
				Insulin II	1.17	1.77	2.98	1.01			Rabbit quiet
12/3	F9	2.5	E_5 and E_6	Basal I	2.13	1.58	4.03	0.74	Nor 0.122		
				Basal II	1.76	1.38	3.36	0.78	2 hr. 0.050		
				Insulin I	2.00	1.39	3.74	0.69	0.072		
				Insulin II	1.12	1.47	2.37	1.28			Rabbit urinated
12/17	H10	2.6	E_5 and E_6	Basal I	1.76	1.30	4.15	0.73	Nor 0.105		
				Insulin I	2.14	1.11	4.97	0.66	2 hr. 0.057		
				Insulin II	1.91	1.56	4.56	0.78	0.048		Rabbit quiet
1924											
2/25	L1	2.3	E11S(2)	Basal I				0.70	Nor 0.122		(Time record lost)
	Control		in pH 2	Insulin I	1.33	0.88	2.70	0.68	2 hr. 0.113		Rabbit quiet
			HCl	Insulin II	1.53	1.05	3.12	0.71	0.009		
4/16	N1	2.2	PS	Basal I	1.77	1.28	3.80	0.74	Nor 0.130		Tremor in insulin I
			with 0.1%	Insulin I	1.68	1.72	3.86	1.00	2 hr. 0.080		
			HCl	Insulin II	1.26	1.51	2.88	1.23	0.050		

4/21	Bw	2.8	P8 with 0.1 % HCl	Basal I	1.71	1.21	2.87	0.71	Nor	0.129	Residuals introduced. Convulsion 1 hr. after removal from box. Slightly restless throughout
				Basal II	1.92	1.42	3.25	0.75	2 hr.	0.033	
				Basal III	1.54	1.15	2.60	0.75		0.096	
				Insulin I	1.43	1.21	2.47	0.85			
				Insulin II	1.71	1.46	2.97	0.85			
4/26	BW	2.8	P9	Basal I	1.64	1.27	2.78	0.76	Nor	0.120	1 Train absorbers leaked
				Insulin I					2 hr.	0.042	
				Insulin II	1.31	1.51	2.36	1.10		0.078	Rabbit quiet
4/28	WY	2.0	P9	Basal I	1.29	0.87	3.03	0.72	Nor	0.125	Absorbers leaked
				Insulin I					2 hr.	0.067	
				Insulin II	1.06	1.16	2.66	1.10		0.058	Rabbit quiet
4/30	B1	2.3	P9	Basal I	1.98	1.43	4.05	0.72	Nor	0.100	Absorbers leaked
				Insulin I					2 hr.	0.076	
				Insulin II	2.39	2.84	5.27	1.20		0.024	Rabbit quiet
5/2	BW	2.4	P9	Basal I	1.53	1.16	3.02	0.76	Nor	0.101	
				Insulin I					2 hr.	0.036	Conv. at end
				Insulin II	1.68	1 36	2.68	0.81		0.065	Limp when blood taken
5/12	B3	2.3	P9	Basal I	1.12	0.96	2.30	0.76	Nor	0.104	
				Insulin I	1.71	1.19	3.49	0.70	2 hr.	0.060	
				Insulin II	1.47	1.19	3.09	0.82		0.044	
				Insulin III	1.65	1.19	3.37	0.72	4 hr.	0.077	Rabbit slightly restless
				Insulin IV	1.59	1.16	2.38	0.72			
5/21	WY	1.9	E(29)-30-S	Basal I	1.29	0.87	3.03	0.72	Nor	0.141	Rabbit in fight before— badly bitten and torn. Slightly restless in insulin I
				Insulin I	1.68	1.28	4.21	0.76	2 hr.	0.058	
				Insulin II	1.59	1.25	4.00	0.79		0.083	

TABLE 1—*Concluded*

DATE	ANIMAL NUMBER	WEIGHT	PREPARATION	PERIOD	O_2 PER HOUR	CO_2 PER HOUR	CALORIES PER KILO PER HOUR	R.Q.	BLOOD SUGAR	URINARY NITROGEN PER HOUR	REMARKS
1924		*kilos*			*liters*	*liters*			*grams*	*grams*	
5/21	WY	1.9		Insulin III	1.83	1.37	4.56	0.75			
				Insulin IV	1.54	1.11	3.81	0.72	4 hr. 0.074		
5/23	T1	2.8	E(29)-	Basal I	1.83	1.33	3.09	0.73	Nor 0.103	Nor 0.048	
			30-S	Insulin I	2.05	1.35	3.39	0.66	2 hr. 0.032	2 hr. 0.045	
				Insulin II	1.53	1.29	2.23	0.84	0.071	4 hr. 0.065	Blood in 3rd urine specimen. Rather restless throughout
				Insulin III	1.89	1.23	3.12	0.65			
				Insulin IV	1.77	1.41	3.03	0.79	4 hr. 0.062		
5/26	G1	3.6	E(29)-	Basal I	1.86	1.40	2.44	0.76	Nor 0.106	Nor 0.078	Tremors through last ⅓ of insulin I and all of II. Graphic records lost for III and IV
			30-S	Insulin I	1.98	1.40	2.53	0.63	2 hr. 0.048	2 hr. 0.072	
				Insulin II	1.48	1.44	2.02	0.95	0.058	4 hr. 0.097	
				Insulin III	1.77	1.58	2.41	0.90			
				Insulin IV	2.30	1.63	2.99	0.71	4 hr. 0.054		
10/22	B4	3.4	E(29)-	Basal I	2.24	1.79	2.64	0.79	Nor 0.147		
			30-S	Basal II	2.19	1.68	1.99	0.77	2 hr. 0.063		
				Insulin I	2.49	1.95	2.50	0.78	0.084		
			30-S	Insulin II	2.40	2.03	2.54	0.85			
10/29	T2	3.6	E(29)-	Basal I	2.09	1.59	2.76	0.76	Nor 0.159		
			30-S	Insulin I	2.27	1.66	2.98	0.73	2 hr. 0.076		
				Insulin II	2.08	1.82	2.83	0.88	0.083		

11/10	G1	4.5	E(29)-30-S	Basal I	2.83	2.06	2.97	0.73			Nor	0.065	
				Insulin I	2.62	2.18	2.82	0.83	Nor	0.127			
				Insulin II	2.24	1.94	2.43	0.86			2 hr.	0.079	
11/12	T2	4.0	E(29)-30-S	Basal I	2.15	1.62	2.54	0.75			Nor	0.141	Very difficult to get blood not enough for basal sugar. Also difficulty in giving insulin
				Insulin I	1.80	1.33	2.13	0.74					
				Insulin II	1.86	1.45	2.23	0.78	2 hr.	0.097	2 hr.	0.138	
11/26	G4	3.5	E(29)-30-S	Basal I	1.95	1.45	2.64	0.74	Nor	0.120	Nor	0.037	
				Insulin I	1.84	1.32	2.47	0.72	2 hr.	0.102			
				Insulin II	1.82	1.57	2.54	0.86		0.018	2 hr.	0.051	
11/28	G5	3.5	49	Basal I	2.13	1.59	2.89	0.75	Nor	0.115			
				Insulin I	2.18	1.64	2.95	0.75	2 hr.	0.052			
				Insulin II	1.27	1.73	1.99	1.35		0.063			Rabbit urinated
				Insulin III	1.76	1.32	2.38	0.75	4H	0.063			
				Insulin IV	1.78	1.35	2.42	0.76					

In thirteen of the seventeen experiments there was an increase in CO_2 over the basal in the second period. In three experiments there was practically no change while in two there was a decrease.

Calculations made from the figures on the "average rabbit" reveal the fact that if there were no O_2 change from the basal at the peak of the quotient curve (second insulin period), the CO_2 increase alone would have given an R.Q. of 0.91. If the CO_2 had not increased, and decrease in O_2 alone had resulted, the R.Q. would have been 0.815. The CO_2 change over the basal at the maximum, therefore, was more significant than the O_2 change. Considering the total change in the gases, the sum of the two insulin periods, the CO_2 divergence from the basal is larger also than the O_2 divergence.

In a few experiments (e.g., B1, April 30) there is an increase in O_2 at the peak of the R.Q. curve, the R.Q. in this case rising as the result of an increase in CO_2 output and in spite of the O_2 rise. The rabbit was quiet throughout.

The results as tabulated show considerable constancy in the basal quotient, giving a basal average of 0.75 (fig. 1). After the injection of insulin, there is in the average for seventeen animals no change in the R.Q. during the first hour and a quarter. There is, however, a sharp rise in the period which terminates two hours after the insulin injection. The quotient rises to 0.98 in the average of all the animals; to 0.95 in the average for the five rabbits which were studied longest. At this point some time is lost, about half an hour in all, by the removal of the rabbit for a blood and urine sample and the preliminary period which again must be run. At the period terminating three hours after insulin, the R.Q. in the average for the five rabbits is again at 0.75 and at three and three quarter hours remains still at this level.

The O_2 in the total average rises from 1.74 to 1.93 liters per hour the first period but declines sharply to 1.60 during the second period. The course for the five rabbits is very similar and in the following two hours there is a return to slightly above the basal.

The CO_2 shows an average increase for all the animals following insulin injection, both the first and second periods, but for the five it rises significantly only the first hour and returns slightly toward the basal in the third and fourth periods.

The heat production being based on the O_2 as well as on the R.Q. follows a course which is a sort of mean between these two curves, rising above the basal the first and third periods after insulin, but falling slightly below for the five rabbits in the second when the oxygen absorption was lowest.

From an average normal blood level of 0.121 gram, there occurs a fall, coincident with the R.Q. rise, to 0.059 gram per 100 cc. of blood. This

drop is approximately the theoretical result of one R.U., that being the dose aimed at in each case. The fall is practically the same for the five rabbits the first two hours and at the end of four hours is already rising, at a slower rate toward normal.

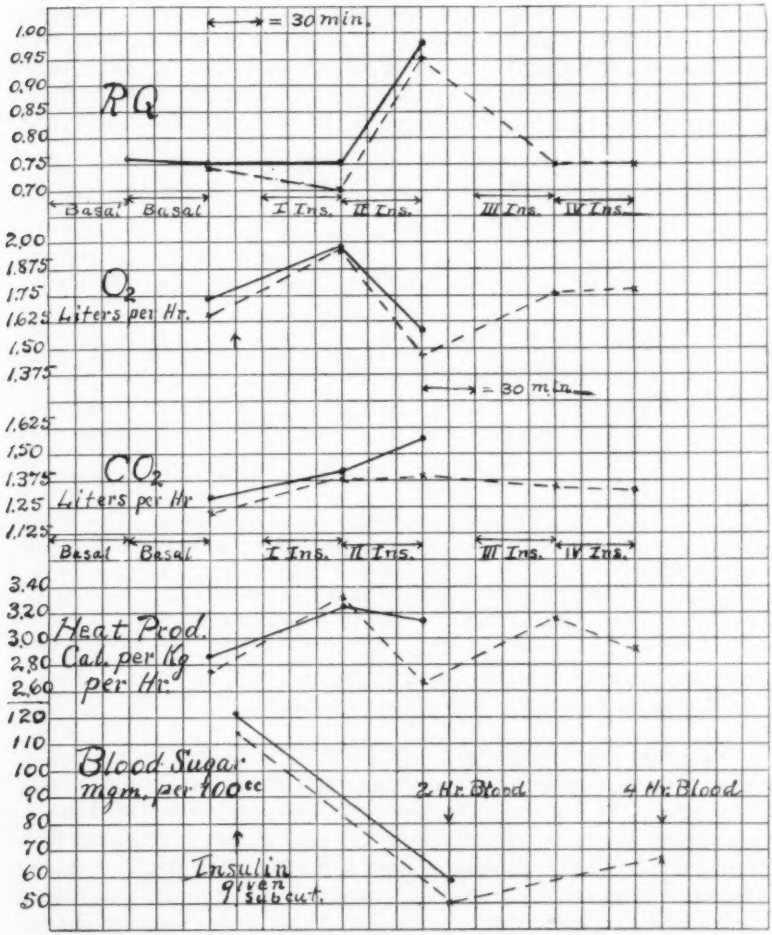

Fig. 1. Showing average results of the effect of insulin on respiratory metabolism. Continuous line—average of seventeen rabbits studied for two hours after giving insulin. Discontinuous line—average of five rabbits studied for four hours after giving insulin.

The change in distribution of foodstuffs metabolized under insulin. What is the effect of insulin upon the character of the combustion process? In table 2 are shown the distribution of calories as computed by the Zuntz and Schumburg method from the nitrogen excretion, the oxygen absorption and the respiratory quotient for two rabbits separately, T1 and G1, and for the average result for the five rabbits which were studied longest in the respiration chamber. Inspection of this table shows clearly that there has been a shifting of the "incidence of metabolism" as Dale (1923) has expressed it, from fat to carbohydrate; for example, a change from 0.02 gram carbohydrate and 0.775 gram of fat per hour in the basal period

TABLE 2

Distribution of calories and grams foodstuffs metabolized under the influence of insulin

RABBIT	DATE	PERIOD	CALORIES FROM				GRAMS		
			Protein	Carbohydrate	Fat	Total	Protein	Carbohydrate*	Fat†
T1	5/23	Basal	1.27	0.08	7.17	8.52	0.30	0.021	0.755
		Insulin II	1.19	3.12	3.03	7.34	0.28	0.83	0.32
G1	5/26	Basal	2.06	0.55	6.05	7.93	0.49	0.148	0.64
		Insulin II	1.90	5.32	0.0	7.22	0.45	1.423	0.0
G1	11/10	Basal	1.72	0.55	10.95	13.22	0.41	0.21	1.15
		Average Insulin I and II	2.09	5.18	4.74	12.01	0.49	1.38	0.50
Average of 5 rabbits B3, WY, T1, G1 and G5, studied 4 hours after insulin		Basal	1.24	0.77	5.66	7.68	0.29	0.21	0.60
		Insulin I	1.32	0.0	7.60	8.92	0.31	0.0	0.80
		Insulin II	1.32	5.10	0.56	6.98	0.31	1.36	0.06
		Insulin III	1.72	0.79	5.81	8.32	0.41	0.21	0.61
		Insulin IV	1.72	0.56	6.07	8.35	0.41	0.15	0.64

* Calculated as glucose, heat value 3.74 calories.

† Calculated as "animal fat," heat value 9.5 calories.

to 0.83 gram carbohydrate and 0.32 gram of fat in the second insulin period, with rabbit T1. In the average result for five rabbits there is a change from 0.21 gram carbohydrate and 0.60 gram of fat per hour in the basal period to no carbohydrate and 0.80 gram of fat in the first insulin period. In this period the effect of insulin in the first hour apparently was to wipe out even the small amount of carbohydrate combustion which already existed. But in the second period there is a sharp change from no carbohydrate to 1.36 grams and from 0.80 gram of fat to 0.06 gram. In the third and fourth periods the average metabolism of these five rabbits returns to substantially the same level as existed in the basal period before insulin.

Our usual conception of such a shifting in the metabolism from fat to carbohydrate is that it causes a higher respiratory quotient and that this higher quotient results from increased elimination of CO_2. Probably

TABLE 3

Comparison of change in oxygen content of foodatuffs metabolized with change in oxygen absorption produced by insulin

RABBIT NUMBER	DATE	PERIOD	LITERS O_2 IN MATERIALS OXIDIZED				LITERS O_2 ABSORBED	LITERS CO_2 ELIMINATED	DIFFERENCE IN CO_2	R.Q.
			Protein	Carbohydrate	Fat	Total				
T1	5/23	Basal	0.016	0.008	0.060	0.084	1.83	1.33		0.73
		Insulin II	0.015	0.311	0.026	0.352	1.53	1.29	−0.04	0.84
						Surplus 0.268	0.30 Deficit			
G1	5/26	Basal	0.026	0.055	0.051	0.132	1.86	1.40		0.76
		Insulin II	0.024	0.530	0.0	0.554	1.48	1.44	+0.04	0.95
						Surplus 0.422	0.38 Deficit			
G1	11/10	Basal	0.022	0.076	0.092	0.190	2.83	2.06		0.73
		Average of Insulin I and II.....	0.027	0.516	0.040	0.583	2.43	2.06	±0.0	0.845
						Surplus 0.393	0.40 Deficit			
Average of 5 rabbits (B3, Wy, T1, G1, G5):		Basal	0.023	0.077	0.048	0.148	1.64	1.23		0.74
		Insulin I	0.024	0.0	0.064	0.088	1.92	1.37	+0.14	0.70
						Deficit 0.060	0.28 Surplus			
						More O_2 required 0.204				
						Total 0.264				
		Insulin II	0.024	0.509	0.005	0.538	1.47	1.38	+0.01	0.95
						Surplus 0.450	0.450 Deficit			
		Insulin III	0.031	0.079	0.049	0.159	1.78	1.34	−0.04	0.75
						Deficit 0.379	0.31 Surplus			
						Less O_2 required 0.033				
						Net Deficit 0.346				
		Insulin IV	0.031	0.054	0.051	0.136	1.79	1.33	−0.01	0.75
						Deficit 0.023	0.01 Surplus			

the paper of Bleibtreu (1901) confirming Pflüger's view as to the conversion of carbohydrate to fat and showing an excess of carbon dioxide in this process is responsible for this conception. Liebig's view was that such a conversion involved a diminished absorption of oxygen. It

appears from what follows that a shifting from fat to carbohydrate may be brought about by diminished absorption of oxygen. A recent erroneous conception is that the combustion of more sugar under the influence of insulin necessarily increases the total absorption of oxygen. Kellaway and Hughes (1923) have fallen into this error. Minkowski (1924) is among the few who have detected the fallacy of this reasoning. "Why should the O_2 consumption not decrease," he says, "when the energy requirements can be covered by the oxygen-rich carbohydrate" instead of the oxygen-poor fat?

Minkowski's query suggested to the writers the idea of comparing the change of oxygen absorption with the altered oxygen content of foodstuffs metabolized. Table 3 shows the results of this computation for three experiments separately and for the average of the five rabbits which were studied longest.

The amount of O_2 necessary to oxidize any food substance or any assortment of food substances is the difference between the O_2 required to convert all of its C and H to CO_2 and H_2O on the one hand and the O_2 already present in the food on the other. A rise in the R.Q. caused by a drop in the oxygen should, if it denotes combustion of carbohydrate, just balance the difference between these two amounts calculated for two different periods—a basal before insulin and another following insulin when the typical effect is most manifest.

The simplest case is presented where there is no change in CO_2 elimination as in the case of rabbit G1 November 10 of table 3. Here we have contrasted the basal period with an R.Q. of 0.73 with the average of the first and second insulin periods showing an R.Q. of 0.845. The table shows the O_2 contained in the different foodstuffs oxidized, the total oxygen thus available, the O_2 absorption, the CO_2 elimination, etc. It happens in this instance that the excess of oxygen available in the foodstuffs oxidized per hour in the two insulin periods just balances the diminished oxygen absorption indicated in the table as "deficit." It follows that the total requirement of oxygen to convert the C and H in the foodstuffs metabolized before and after insulin to CO_2 and H_2O is the same, as nearly as this can be calculated without knowing the exact composition of the body fat. This is shown in the following tabulation:

	From table 2 *Grams*	*C*		*H*		*Liters O_2 required*
		% C	*grams C*	*% H*	*grams H*	
Basal Period	Prot. $0.41 \times$	41.5[1] $=$	0.170	4.4[1]	0.0180	
	C. H.[2] $0.21 \times$	40.0 $=$	0.084	6.6	0.0138	
	Fat $1.15 \times$	76.54[3] $=$	0.880	12.01[3]	0.138	
		Total	1.134		0.170	
	Liters O_2 required		2.118		0.951	Total 3.069

[1] Loewy (1911)
[2] Calculated as glucose
[3] Johansson (1910)

	From table 2 Grams		C % C	C grams C	H % H	H grams H	Liters O_2 required	
Average of	Prot.	0.49 ×	41.5	= 0.203	4.4	0.0216		
Insulin I	C. H.	1.38 ×	40.0	= 0.552	6.6	0.091		
and II	Fat	0.50 ×	76.54	= 0.383	12.01	0.06		
			Total	1.138		0.1726		
	Liters O_2 required			2.126		0.966	Total	3.092
	Difference between Basal and Insulin I and II							0.023

Of course this is only another way of proving the metabolism figures as presented in table 2, for the values (or approximately these values) employed above are all implicit in the Zuntz and Schumburg method based upon the heat value of nitrogen excreted and upon different thermal quotients for the oxygen at different levels of the non-protein R.Q. But it visualizes just what is implied in such an alteration of the metabolism as is presented in table 2. Included in the O_2 requirement for the insulin periods is 0.393 liter of O_2 already contained in the food. The oxygen absorption has diminished 0.400 liter (see table 3). Apparently what insulin has done in this instance is to set free this oxygen contained in carbohydrate so that it participates in the oxidation. Actually the sugar molecule is probably disrupted by the insulin in some way favorable to complete oxidation, in the course of which the O_2 of the carbohydrate is made available.

With rabbits T1 and G1 (for May 26) the agreement between the extra oxygen available in the foodstuffs oxidized and the diminished oxygen absorption is nearly as good. In each case there is only a slight change in the CO_2 elimination. Computation of the O_2 requirement for complete conversion of C and H would exhibit but a negligible difference between the two periods.

A more complex situation is presented when we analyze the whole result of insulin action as it is revealed in the average of five rabbits, B3, Wy, T1, G1 and G5. Table 2 exhibits the altered metabolism. In table 3 can be seen the comparison between deficit or surplus, as the case may be, of oxygen available in the foods metabolized, with the altered absorption. Computing the oxygen requirement as above we find:

	From table 2 Grams		C % C	C grams C	H % H	H grams H	Liters O_2 required	
Basal	Prot.	0.29 ×	41.5	= 0.120	4.4	0.013		
period	C. H.	0.21 ×	40.0	= 0.084	6.6	0.014		
	Fat	0.60 ×	76.5	= 0.459	12.01	0.072		
			Total	0.663		0.099		
	Liters O_2 required			1.239		0.554	Total	1.793
Insulin I	Prot.	0.31 ×	41.5	= 0.128	4.4	0.014		
	C. H.	0.0 ×	40.0	= 0.0	6.6	0.0		
	Fat	0.80 ×	76.5	= 0.612	12.01	0.096		
			Total	0.740		0.110		
	Liters O_2 required			1.383		0.616	Total	1.999
	Additional for CO_2			0.142, for H_2O		0.062	Total +	0.204

	From table 2 Grams		C % C	C grams C	H % H	H grams H	Liters O_2 required
Insulin II	Prot.	$0.31 \times 41.5 =$		0.128	4.4	0.014	
	C. H.	$1.36 \times 40.0 =$		0.544	6.6	0.090	
	Fat	$0.06 \times 76.5 =$		0.046	12.01	0.007	
		Total		0.718		0.111	
	Liters O_2 required			1.341		0.621 Total	1.962
	Less for CO_2			0.042, more for H_2O		0.005, net change	− 0.037
Insulin III	Prot.	$0.41 \times 41.5 =$		0.170	4.4	0.018	
	C. H.	$0.21 \times 40.0 =$		0.084	6.6	0.013	
	Fat	$0.61 \times 76.5 =$		0.467	12.01	0.073	
		Total		0.721		0.104	
	Liters O_2 required			1.347		0.582 Total	1.929
	Additional for CO_2			0.006, less for H_2O		0.039 net change	− 0.033
Insulin IV	Prot.	$0.41 \times 41.5 =$		0.170	4.4	0.018	
	C. H.	$0.15 \times 40.0 =$		0.060	6.6	0.010	
	Fat	$0.64 \times 76.5 =$		0.490	12.01	0.077	
		Total		0.720		0.105	
	Liters O_2 required			1.345		0.588 Total	1.933
	Less for CO_2			0.002, more for H_2O		0.006 net change	+ 0.004

Referring again to table 3, it is seen that the calculated metabolism for the first insulin period discloses a deficit in the oxygen available in the foodstuffs themselves of 0.060 liter while there is an increased absorption of 0.280 liter. The computation above discloses also an additional requirement for oxygen to convert C and H to CO_2 and H_2O of 0.204 liter. The increased absorption evidently makes up both these quota (0.060 plus 0.204 equals 0.264) and leaves a small margin of 16 cc. The additional requirement for CO_2 is accounted for in the extra elimination of 0.14 liters CO_2.

The altered metabolism in this first insulin period deserves notice. The R.Q. has been depressed in consequence of the larger absorption of oxygen, and notwithstanding the increased elimination of CO_2. This denotes a larger metabolism of fat. The effect on the heat production is to be seen in table 2. Since but little effect on the muscular activity of the animals was to be seen in the continuous graphic records for this period, we are justified in speaking of a *calorigenic action* of the insulin at this early stage (within one hour of injection). Direct calorimetry should confirm this (see below).

In the second insulin period the oxygen absorption decreases sharply thus raising the R.Q. without effect on the CO_2. As before, in the case of rabbit G1, we find for the average of five animals that the extra oxygen

contained in the foodstuffs oxidized, balances exactly the diminished absorption, and the computation of the oxygen requirement for C and H combustion respectively shows a net change of only 37 cc. This comes as near no alteration in the total oxygen requirement as could be expected, considering that the exact composition of body fat cannot be known, and that the water formed in combustion cannot be separated from preformed water. If there is no change in the total oxygen requirement, why should the oxygen absorption decline? Manifestly because the oxygen contained in the carbohydrate brought into metabolism is now available, and is not merely split off in combination with H as H_2O. Table 3 shows that the extra available oxygen is all contained in the carbohydrate. It appears to be no mere coincidence that this is true for if the oxygen absorption had fallen to 1.57 liters instead of to 1.47 liters, the extra oxygen available would not have balanced the decreased absorption so well.

The apparent difference of only 37 cc. in the oxygen requirement only emphasizes the nicety with which the diminished oxygen absorption has offset the surplus oxygen available. It may be fairly assumed that this new oxygen from carbohydrate split up by insulin diminishes the withdrawal of that gas from the blood into the tissues and in the absence of any stimulus to the respiratory center (Eadie, Dickson, Macleod and Pember, 1924) the absorption from the inspired air is diminished merely because the blood is already saturated. Venous blood should therefore be somewhat less "reduced" than previously. Observations on saturation of hemoglobin seem thus far to have been complicated by change in blood volume which make the interpretation difficult (Olmstead and Taylor, 1924).

At this point we should "close the account" temporarily for an interval of thirty minutes intervenes before the beginning of period III after insulin. When again the debits and credits are balanced it is seen that a deficit of 0.379 liter has been incurred through reliance upon a "frozen" physiological security in the form of fat—a much less "fluid" form of asset than carbohydrate which was the main reliance in period II. Outside credits must therefore be absorbed into the system. However, it is found upon looking up "accounts payable" that 0.033 liter less than in the previous period are due. The net liability therefore is only 0.346 liter to apply upon which 0.310 liter have been called in from outside. Period IV immediately follows and again a small deficit seems to have been incurred, which is not improved by the most careful audit possible. If the accounting could have been perfectly continuous from beginning to end, it is probable the books could have been balanced a little more perfectly.

What is that particular hormone in banking operations which enables

a managing director to keep his assets "fluid?" Whatever its name in secular institutions, in the more nicely regulated operations of the animal economy its name is insulin. Without this influence almost the only resource is fat and an organism which must rely upon fat alone is bankrupt. A diabetic has plenty of resources but none of them "fluid."

It is unfortunate that the same evaluation of the results for the entire group of rabbits cannot be made. The protein metabolism must be known and every detail of the respiratory metabolism for each period to make this possible. We have sufficient data on the entire group, however, to make certain that the essential changes in metabolism brought about by insulin hold for the average of all as for the average of the five (see fig. 1).

SOURCE OF THE CARBOHYDRATE BURNED. The study just completed brings to light a convincing reason for the disappearance of glycogen from the liver of the insulinized but otherwise normal animal. For when we compute the amount of sugar which conceivably could come from the body fluids and compare it with the amount of carbohydrate which has been burned we find a great discrepancy. The following table (table 4) makes this clear.

TABLE 4

Comparison of sugar from the blood with total sugar oxidized

RABBIT NUMBER	WEIGHT	BLOOD SUGAR FALL IN 2 HOURS	GREATEST AMOUNT FROM CIRCULATION	SUGAR OXIDIZED IN 1 HOUR	FROM GLYCOGEN
	kgm.	*grams*	*grams*	*grams*	*grams*
T1	2.8	0.071	0.120	0.81	0.69
G1	3.6	0.058	0.125	1.275	1.15
Average of five rabbits.....	2.82	0.063	0.126	1.36	1.24

An allowance of 6 per cent of the body weight was made for the total blood in circulation. The greatest amount which the blood itself could furnish without replenishment is but 8 to 10 per cent of the quantity which has been burned in only one half the time. Even estimating the entire body fluid at nearly 16 per cent of the body weight as Burn and Dale do and assuming that this entire volume is brought rapidly into equilibrium with the blood, we find only 20 to 28 per cent.

The method adopted in this study did not permit observations on the time relations between the respiratory effects and the incidence of blood sugar effect. Others (Krogh, 1923) (Dixon, Eadie, Macleod and Pember, 1924) have found that the first rise in R.Q. occurs at about the time the blood sugar completes its initial fall so that some time before this, we may fairly assume the glycogen stores are already responding to the

demand for more carbohydrate. The blood sugar however begins to fall as a rule immediately and numerous observations in this laboratory confirm those of other authors that within thirty minutes from the time of injection of a rabbit unit the decline is more than half completed. Bissinger, Lesser and Zipf (1923) found that the effect upon the carbohydrate store recoverable from the entire body of a mouse was to be seen within ½ hour, and Lesser (1924) later observed that within this time there was no change in the respiratory quotient.

The results of the present study show that in one half of the cases the CO_2 elimination does perceptibly increase in the first period starting 15 minutes after injection, but that in all but one of these the O_2 absorption simultaneously increases still more so that the quotient is depressed. These changes, producing more heat, have already been interpreted (p. 122) as a stimulating effect. In only a few (e.g., Bw, April 21 and G1, November 10) does the rise in quotient caused by a falling oxygen, so characteristic of the second insulin period, occur in the first. There seems therefore to be a lagging of the final stages of combustion resulting in increased CO_2 or lower oxygen (because that of the carbohydrate is now available) behind the fall in blood sugar, although this cannot be vouched for by the present writers on the basis of coincident observations.

Discussion. Macleod (1923) in his insulin lecture at Edinburgh advocated the view that the oxidation of sugar in the tissues is not augmented under insulin. Dale (1923) on the contrary and Krogh (1923) voiced the opinion concurred in later by Minkowski (1924) and others that the characteristic action of insulin would be found to consist of a sudden shifting of the metabolism from fat and protein to carbohydrate. Macleod (1924) in his later review seems to have adopted this idea. The present findings confirm and amplify it. The key to the situation is the fall in oxygen absorption and its correct interpretation.

Meantime this fact has been reported also by Gabbe (1925) from his experiments on rats. He finds that medium or large doses regularly produce a fall in O_2 absorption by these animals in the first hour, with a return to normal the second or third hour. The CO_2 did not rise. Gabbe emphasizes the fact that increased combustion of carbohydrate does not call for greater absorption of oxygen as assumed by Kellaway and Hughes and Geelmuyden (1923). His results are given as yet only in a preliminary communication. Quite possibly in his completed work he will discover the agreement between the fall in oxygen absorption and oxygen made available by the carbohydrate, which has appeared in this study.

Lesser (1924) has reported continuous respiration experiments upon white mice which had previously received an injection of glucose. When insulin was injected in doses which did not reduce the rate

of oxidation the quotient rose far more than after glucose alone. Lesser does not publish CO_2 determinations but it appears from the oxygen figures that even when no hyperglycemic symptoms were seen the absorption of this gas was considerably reduced. When the carbohydrate combustion was reckoned up it was found that small doses of insulin accelerated combustion more than large. In comparison with chemical analyses of Bissinger, Lesser and Zipf (1923) upon the entire body of mice treated with insulin it appeared that from 30 to 50 per cent more carbohydrate as such vanished from the body when insulin took effect than could be accounted for by combustion or glycogen formation. Lesser admits however that his method of calculating the combustion is merely provisional. Moreover he found that the same discrepancy occurred when no insulin was given.

A similar conclusion regarding the relation of sugar oxidized to sugar disappearing from the system was reached by Burn and Dale (1924) in their work upon eviscerated animals receiving glucose continuously and concurrently with the R.Q. observations. More sugar was lost to view than could be accounted for by combustion. They calculated the oxygen absorption which would be necessary to dispose of all the sugar and found it related to the actual absorption as 1.6 to 1.0 (average of five experiments).[2] This is a much closer agreement than Bouckaert and Stricker (1924) found from their direct calorimetric experiments upon rabbits. Sugar disappearing was more than five times the quantity necessary to account for the total heat loss. Lesser (1925) has made use of all of these experiments in drawing a comparison between the effects of insulin and the carbohydrate metabolism accompanying recovery of muscle as elaborated by Meyerhof (1920 to 1924) and Hill (1920 to 1922). The former has found that when two Mols of lactic acid burn twelve Mols become synthesized again into glycogen and Hill's myothermal experiments in conjunction with Hartree substantially confirm the caloric relationship postulated by Meyerhof. There is as yet no substantial basis for drawing such an analogy, much less for attempting to identify the effect of insulin on resting tissue with the recovery process in muscle after contraction. The best case Lesser can make out is from Bouckaert and Stricker's work which certainly needs confirmation. Other results, his own included, indicate a relationship of carbohydrate disappearing to carbohydrate burned on the molar bases of 1.5–1.7 to 1.0.

[2] Burn and Dale's estimate of body fluid (blood, lymph and tissue fluid) contains at least an error of 10 per cent in the example given by them which, when corrected in the only experiment (no. 9) of their series where data would admit of correction, considerably alters the discrepancy between observed and calculated oxygen. The error consists in an oversight of increased combustion caused by accelerated injection. The authors admit that the method of estimating the total fluid into which the sugar disappears is crude. They did not estimate the probable error of such a method.

Much more promising would seem to be the clue established by Hopkins and Winfield (1915) and Foster and Woodrow (1924) that the pancreas furnishes a substance which has a controling action on the carbohydrate breakdown of muscle. This substance inhibits the anaerobic formation of lactic acid in muscle and certainly is not identical with insulin. They suggest that carbohydrate metabolism may be grouped into two main subdivisions, that of the body as a whole under the control of insulin, and that of muscle under the control of the new pancreatic hormone.

The results reported above seem to support the distinction made by Foster and Woodrow, since the amount of extra carbohydrate oxidized in place of fat exceeds by far the amount which by the most liberal allowance can have disappeared from the body fluids. The substitution of carbohydrate for fat in the combustion of the fasting rabbit under insulin treatment, as demonstrated by a complete metabolism experiment, stands on quite another basis from the uncertainties involved in chemical estimation of the total carbohydrate which has disappeared from the system, whether in the normal or the insulinized animal.

The rate of oxidation bears a direct relation to the sugar concentration in the eviscerated animal, as Burn and Dale have clearly shown. It must bear a relation also to the concentration of insulin; but the law governing this relationship cannot be formulated until much more delicate methods are at hand for estimating the insulin in circulation. The rate of combustion assuredly is something quite different from the rate of disappearance of sugar from the blood. The average of five rabbits (see fig. 1) displays a reduction in blood sugar of 23 mgm. per kilo in two hours, and an augmented rate of combustion of sugar in the first two periods of 200 mgm. per kilo per hour. The average of all the seventeen rabbits is 22.6 mgm. fall in blood sugar per kilo for two hours and by an approximate calculation, 270 mgm. combustion of sugar per kilo per hour.

The significance of the effect upon heat production should not be overlooked. In every instance where the exact comparison could be made (table 2) between basal and insulin periods, it is seen that the first effect is increased heat production, as calculated and in the second period where carbohydrate substitution comes in reduction below the basal level. Noyons, Bouckaert and Sierens (1924) made direct measurements of heat loss in rabbits after insulin treatment and found either no change or a slight increase. The rectal temperature of the animal sank. When it went as low as 34°C., the heat production also declined. When the latter fell as much as 30 per cent the animal died. All their experiments seem to have been made with large doses of insulin.

When the experiments reported above were performed, the apparatus was not equipped to make coincident observations on body temperature.

Readings made at the time of taking blood, with ordinary mercury thermometers, did not give any such change as Noyons and his colleagues found. In fact out of five carefully controlled tests made upon animals treated with good insulin preparations, but not placed in the respiration chamber, only one gave a small drop (0.3°C) in two hours although the sugar reduction in the blood was as much as expected (average of 51 mgm.). On another occasion a drop of 0.5°F. was observed with a particularly pure insulin. From the change in heat production noted early in the experiments, it was anticipated that a coincident decline in body temperature at the height of the glucopyretic effect would be found but out of more than a dozen tests there was either no change or a slight rise in all but two, as mentioned above. Quite possibly the difference between our observations and those of other authors consists in the size of dose employed or possibly, also, in the purity of the product. The subject is reserved for further study in connection with direct calorimetry.

SUMMARY AND CONCLUSIONS

1. Respiration experiments by the closed circuit method employing an apparatus which gave perfect control checks are reported upon twenty fasting rabbits, seventeen of which received an injection of insulin subcutaneously which reduced the blood sugar an average of 62 mgm. in two hours. The aim was to avoid preconvulsive excitability. Movements of the animal were recorded graphically and in only two instances were there any signs of increased excitability.

2. No correlation could be established between the extent of change in the respiratory metabolism and the amount of change in the blood sugar.

3. One or two basal periods of 45 to 60 minutes each were obtained before giving insulin and two periods of at least 45 minutes each in all cases, and four periods in five cases were obtained after insulin. An interval of fifteen minutes intervened after giving insulin before starting the first respiration period. An interval of thirty minutes intervened between the second and third periods for drawing blood and for reëstablishing equilibrium in the apparatus.

4. The results, shown graphically, are: *a.* No rise, but often a fall, in respiratory quotient the first period (up to one hour after insulin). *b.* A sharp rise to 0.98 (average of seventeen animals) in the second period. *c.* In the third period a rapid return to the pre-insulin level which persists also in the fourth period. *d.* The oxygen absorption on the average rises the first hour and falls considerably below the pre-insulin level the second hour. It returns to slightly above normal in the third and fourth hours (average of five). *e.* The CO_2 elimination rises the first hour and in the average for all the animals rises still further the second so that the

sharp rise in R.Q. is the result of changes both in the O_2 and the CO_2. In the average of the five studied longest the CO_2 does not rise further in the second period and falls toward but does not quite reach the pre-insulin level in the third and fourth periods. *f.* The heat production rises sharply in the first hour only to fall again in the second. In the average of the five studied longest, the fall in the second is greater than the rise in the first period; but it rises again in the third and terminates in the fourth exactly at the pre-insulin level (end of three and three-quarters hours).

5. Calculations of the metabolism by the Zuntz and Schumburg method on several animals in which the urinary nitrogen was known shows in the first hour after insulin an average increase in the metabolism (chiefly of fat) (calorigenic action amounting to 16 per cent). In the second period, terminating at one and three-quarters hours after insulin, there is an abrupt change in the "incidence of metabolism" (Dale) from fat to carbohydrate. For example, from no carbohydrate to 1.36 grams per hour and from 0.8 gram of fat to 0.06 gram in passing from the first to the second insulin period (average of five rabbits). These changes are believed to be the characteristic action of insulin (glucopyretic effect) whether in normal or diabetic animals (citation of previous work).

6. Diminished oxygen absorption in the second period is not due to depression but to the fact that additional oxygen is made available for combustion (heat production) through a change to an oxygen-rich foodstuff. The diminished absorption in several instances exactly balances the additional oxygen thus made available.

7. Sugar burned under the influence of insulin is far greater than can be accounted for by the disappearance of sugar from the blood or from the blood, lymph and tissue fluid at the most liberal estimate. Hence the disappearance of glycogen from the liver and other organs of normal animals, as reported by many authors. There is no occasion to search frantically for an unknown intermediary substance into which the sugar is transformed, nor is there any basis for identification of the action of insulin with the mechanism for recovery of muscle after contraction (Meyerhof-Hill).

8. The reduction of heat formation, as calculated at the height of the glucopyretic action, would lead one to expect a fall in body temperature; but this has not been confirmed in the present observations, probably due to the small dose of insulin employed.

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BIURET-FREE INSULIN

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A form of insulin which does not give a biuret reaction has been repeatedly prepared in this laboratory. It is obtained readily only by the perfusion method but has been prepared several times also by the reversed percolation method (Clough, Allen and Murlin, 1924). Numerous attempts have been made to remove all of the protein from insulin prepared by ordinary extraction but without avail. An additional difficulty has been the extreme sensitiveness of the biuret-free insulin to reducing reagents and other substances which deprive it of its characteristic physiological property. A brief paper has already been published (Allen, Piper, Kimball and Murlin, 1923) giving the essential steps for the preparation of biuret-free insulin and a few statements given regarding its properties. The present paper amplifies the former description.

It was early observed that crude insulin prepared by perfusion contained much less protein than when prepared by extraction by any of the methods tried in this laboratory. The crude perfusate could be injected into animals without any sign of toxic properties. This was in sharp contrast with the crude aqueous acid extract (Murlin, Clough, Gibbs and Stokes, 1923). It was also early observed that the proteins had a different iso-electric point. This fact is well illustrated by the following experience. Starting with a pH of 4.1,[1] which had been found to be a satisfactory reaction for the first precipitation of proteins by neutralizing the acid used in ordinary extraction, no precipitate is obtained. There is only a slight turbidity. But by continuing the addition of alkali a well-defined precipitate is formed at a pH of 5.85. If this precipitate is removed by filtration and the filtrate is further treated with sodium hydrate up to pH 7.0 no other material is precipitated. On the other hand, if the precipitate which comes out at pH 5.85 is left in the product and alkali is added to pH of 7.0, the character of the precipitate is changed quite markedly. It becomes more flocculent and settles out of the solution much more quickly than previously. If more alkali is added so as to make the preparation alkaline to litmus, this precipitate at 5.85 redissolves. The above-mentioned facts

[1] Determined electrometrically.

have definite effects upon the insulin content of the preparations. This will be seen at once from a study of table 1.

Perfusate 40 was divided into two equal portions, one 40-A, was adjusted to a pH of 4.1. There was no change in this portion other than a slight increase in turbidity. The other portion, 40-B, was brought to a pH of 5.85. A fine white precipitate was formed. This was removed by filtration through paper and the filtrate, which was a clear, sparkling liquid, was readjusted to pH of 4.1, the reaction usually employed in physiological testing. The precipitate was treated with distilled water and tested on rabbits. The resulting tests indicated that the precipitate contained but little potency. Filtrates from 40-A and 40-B were likewise tested on rabbits in sufficient number to give a good assay. These tests showed an average yield of 357 rabbit units per kilo of pancreas for 40-A and an average yield of 492 R.U. per kilo for 40-B. The experiment indicates

TABLE 1

Influence of reaction upon perfusates

(All tests in same equivalent dose)

PREPARATION NUMBER	ISOELECTRIC PRECIPITATION AT pH	AVERAGE BLOOD SUGAR CHANGE, 2 HOURS	R.U. PER KILOGRAM* PANCREAS, AVERAGE YIELD
		mgm.	*R.U.*
40-A	4.1	−63	357
40-B	5.85	−86	492
41-A	4.1	−29	166
41-A-2	4.1 first 5.85 later	−63	369
41-B	5.85 at once	−50	288
41-C	8.0	−54	314

* For method of calculating yield, see Clough, Allen and Murlin (1924).

that 30 per cent of the potent material was tied up with the acid metaprotein in the case of 40-A and that it was freed in the portion 40-B.

The following perfusate, 41, was studied in a similar and further manner. Made exactly like no. 40 except that the amount of pancreas perfused was about two and one-half times as great, the volume of the perfusate in this instance nevertheless was the same. It was divided into three portions. The first, 41-A, was adjusted to pH 4.1 as in the case of 40-A. Sufficient tests showed an average yield of 166 R.U. per kilo. This portion, after having stood for eight days was adjusted to pH of 5.85, filtered and the filtrate returned to pH 4.1, whereupon it gave an average yield of 369 R.U. which is an increase of 120 per cent over the former test of 41-A. This product is designated in the table as 41-A-2. The precipitate from 41-A-2, when adjusted to 5.85 gave no positive test.

The second portion of the preparation, 41-B, was adjusted to pH of 5.85,

immediately, as in 40-B. The product when tested in duplicate showed an average yield of 288 R.U. The "5.85" precipitate was found to contain 223 R.U. (This point will be brought up later under the explanation of table 2.) As in the case of 40-A and 40-B, 41-B shows that about 74 per cent of the active substance was in some way bound up in the precipitate of 41-A.

The third portion, 41-C, was at once made neutral to phenolphthalein, or a pH of 8.0. A heavy flocculent precipitate was formed and settled out of solution very rapidly. The product was filtered and the filtrate brought back to 4.1. The average yield was 314 R. U. per kilo which is similar to that of 41-B. The precipitate, however, contained no potency whereas that of 41-B contained almost as much activity as did the preparation itself.

TABLE 2

Distribution of insulin in perfusates. Recovery of insulin from precipitates

PREPARATION NUMBER	AVERAGE BLOOD SUGAR CHANGE, 2 HOURS	R.U. PER KILOGRAM PANCREAS, AVERAGE YIELD	TOTAL YIELD	
			Calculated	Actual
	mgm.	*R.U.*		
41-B*	−50	289	512	
41-P†	−39	223		
41-B + P	−84	477		477
44-B	−64	363	712	
44-P	−61	349		
44-B + P ½ dose	−67	760		760
45-B	−60	340	586	
45-P	−43	246		
45-B + P ½ dose	−59	675		675

* B signifies a preparation adjusted to pH of 5.85.

† P signifies a preparation made by reëxtracting the precipitate from B with HCl (pH 2.0).

The iso-electric point of the proteins with which insulin is associated in the perfusate therefore lies at about 5.85 (electrometric) If the precipitate is large, i.e., resulting from a fairly concentrated solution, it should be reëxtracted. The significance of this latter point will be clearly seen in table 2.

As has already been mentioned, the "5.85" precipitate from 41-B contained about as much potency as the filtrate 41-B. This precipitate is designated as 41-P in table 2. 41-B showed an average yield of 289 R. U. per kilo and 41-P 223 R.U. per kilo. The sum of the two is 512 R.U. per kilo. The average yield after combining a distilled water extract of 41-P with the original from which the precipitate came, was found to be 477 R.U. per kilo. This combination is represented in the table as 41-B plus P. There is a difference, therefore, of about 6.8 per cent between the actual yield and that calculated.

Similar results were obtained in the case of perfusates 44-B and 45-B. 44-B had an average yield of 353 R.U. per kilo while the precipitate 44-P showed an average of 349 R.U. The "5.85" precipitate in this case was reëxtracted in hydrochloric acid solution of pH 2 in place of distilled water as in 41-B. The insoluble material was filtered off and discarded, the filtrate containing the potency. The combination of 44-B and 44-P or "44-B plus P" gave an average yield of 760 R.U. per kilo, the calculated yield being 712 R.U. (363 plus 349). The difference between the actual and calculated yields in this instance is 6.78 per cent.

For perfusate 45-B and 45-P the yield was 340 R.U. and 246 R.U. respectively. The product 45-P was prepared the same as 44-P, namely, reëxtracted with HCl solution of pH 2.0. The combination "45-B plus P" showed an average yield of 675 R.U. per kilo as compared to the calculated 586 R.U. per kilo.

Method of purification. The foregoing observations thus established points of procedure which became fixtures in the method for the purification of the insulin preparations secured by perfusion. Further purification is accomplished in a manner similar to that utilized in the purification of simple extracts and more particularly percolates, which process in reality resulted from the perfusate procedure. It was found, however, that the hypoglycemic substance could not be precipitated by amyl alcohol as in the case of the ordinary extraction method. A precipitate is formed but it is much less voluminous than the corresponding precipitates of extracts. It was found to contain no potency.

It is significant for the question of the nature of insulin that a method of precipitation which gives a very highly potent preparation when applied to crude extracts containing much protein should not succeed when applied to perfusates containing relatively little protein.

Since the method of precipitation used with simple extractions could not be applied to perfusates, this step had to be omitted. It was found that salting out with sodium chloride, in the ratio of 35 grams of the salt per 100 cc. of solution as with simple extracts, caused complete removal of the insulin complex, none whatsoever being left behind in the filtrate. The salt is removed for the most part by subsequent treatment with alcohol of 70 and 80 per cent. The precipitate allowed to dry on the filter paper is extracted with 70 per cent alcohol and the insoluble substance which consists of large amounts of salt and protein is removed by filtration. The filtrate is next evaporated to dryness in vacuo and the dry residue again extracted with alcohol (80 per cent) using one-half as much alcohol as was used in the previous step. The salt and some protein is filtered off as before, the filtrate being brought to dryness in vacuo. Again, the residue is treated with 80 per cent alcohol diminishing the amount to one-half that taken in the step before. The liquid is decanted from the insoluble

substance and dried as in the previous instance. This process is repeated three more times, each time the volume of alcohol used being diminished to one-half of the preceding volume. At the end of the sixth fraction, the dry residue is treated with sterile water. The resulting product is always turbid but there is no precipitate which can be removed by filtration or centrifugation. The acidity of the preparation is adjusted to a pH of 4.1 whereupon a fine precipitate is formed. This is removed by centrifugation. The liquid is decanted and the precipitate treated with sterile water. The precipitate is insoluble in neutral water and contains potency. The liquid from which this precipitate came also is potent although on several occasions this portion has shown no positive tests, all of the active substance being in the insoluble state.

The insoluble, A-biuret product. The A-biuret product is insoluble in acid solutions and in water, as indicated, but it is soluble in the very faintest trace of alkali. A preparation of amphoteric nature, however, was once secured, being soluble in weak acid as well as alkali. That the insolubility is really a property of the active material and not merely of a precipitate of inactive protein to which the insulin has adhered is indicated by the failure of all attempts to wash the precipitate free of insulin and by the fact that very often the supernatant fluid obtained by centrifugation contains no potency. The insolubility in water or weak acid does not interfere with rabbit testing for the biuret-free insulin immediately goes into solution in the body fluid.

It does not give a biuret, xanthoproteic, Millon's Hopkins-Cole or Molisch test. Also, the $AgNO_3 - HNO_3$ test for chloride is negative. The absence of the above qualitative tests for protein indicates that insulin is *not* a protein as was formerly believed and is still maintained by Shaffer (1924), by Dudley (1923) and by Dodds and Dickens (1924) in particular Shaffer who strongly believes that insulin is a protein, based on his observations of an "iso-electric" product which gives all of the characteristic protein reactions, suggests that we use solutions which are too dilute in making our protein tests. In order to meet this criticism, an insoluble product, which came from perfusate 47, was evaporated to dryness and the entire dry residue dissolved in the smallest amount of alkali. The biuret test was negative notwithstanding that nearly 100 clinical units were present. The test when applied in the same manner to an equivalent amount (5.1 mgm.) of pure egg albumin and Witte's peptone, respectively, was very strongly positive; 0.4 mgm. of each of these substances gives a distinctive test.

The protein-free product was first secured from perfusates and can be obtained invariably from this particular method of extraction. However, if a perfusate contains much protein substance to begin with, the final insoluble product will, as a rule, give a very faint biuret test. It can be

freed from the protein material by further treatment with alcohol, as before, filtering off and discarding the insoluble material and evaporating the filtrate to dryness. Some of the potent substance is lost, but the most of it is present in the resulting A-biuret product. The protein-free preparation of 40-A (table 3) was subjected to the above treatment. Others have been purified in this manner likewise. Many times we have tried to prepare the A-biuret insulin from percolates and simple extracts. The method, from the salt precipitate on, was the same as used for the perfusates. It was obtained on a few occasions from percolates but more frequently the final product contained protein. Very often a final insoluble product answering the qualitative chemical tests was obtained but when tried for potency none was present. The protein-free preparations of perfusates have been found to be very unstable, the activity being lost spontaneously within a week and very often within a few days when kept at a pH which preserves ordinary insulin. The insoluble product, above discussed, is a much purer product than the ones used in those experiments.

Table 3 gives the results of various attempts to convert all of the crude insulin into the biuret-free form. Comparing the yield of these preparations with the yield of the original preparation from which they came, it will be seen that the greatest total yield of the A-biuret product, in the case of perfusates, is over 100 per cent and for those from the percolates the greatest is 51 per cent. A curious fact is that when a perfusate (no. 42) and extract (no. 3) were combined, the total yield of the resulting protein-free product was 95 per cent. The insulin compound was practically all converted into the insoluble A-biuret product. Whether the presence of the perfusate was instrumental or not in making possible the conversion of the insulin from the extract into the insoluble compound is a question that cannot be satisfactorily answered at present. In the following combination, which consisted of a perfusate (no. 43), a percolate (no. 4-B), and an extract (no. 8), this does not appear to have been the case. Here the total yield of the resulting protein-free compound is only 24 per cent. Comparing the number of "rabbit units" per kilo, it is seen that the yield for the biuret-free product is practically the same as the original yield of the perfusate. Since experience has shown that this preparation is more readily secured from perfusates than from either percolates or extracts, one might conclude that the product came from the perfusate alone, but such a conclusion cannot be considered as proved.

Discussion. Protein-free insulin seems to have been obtained first by Zuelzer, since in the specification to the patent applied for by him to the U. S. Government in 1908 and granted in 1912, he describes a substance derived from the pancreas by extraction with alkaline solutions which after treatment with alcohol gave "none of the protein reactions" but caused lowering of the blood sugar. Since Banting and Best's work Wallis

(1922) also has claimed anti-diabetic properties for a product obtained by extraction with alcohol which is free of proteins and of peptones, cholesterol, histidin, histamin and cystin. It is said however to contain polypeptides and some lipoid material. Best and Macleod (1922) at first thought they had a protein-free form obtained by alcoholic extraction

TABLE 3

Biuret-free insulin preparations

(Derived from perfusates, percolates and other extracts)

PREPARATION NUMBER	PANCREAS WEIGHT RATIO	AVERAGE BLOOD SUGAR CHANGE, 2 HOURS	R.U. PER KILOGRAM PANCREAS, TOTAL YIELD	PER CENT OF TOTAL IN BIURET-FREE EXTRACT
Perfusates				
		mgm.	*R.U.*	*per cent*
39	5.0:2K	−71*	202	73
39 biuret-free	5.0:2K	−52*	149	
40-A	2.5:2K	−63	357	114
40-A biuret-free	2.5:2K	−71	407	
41-B + P	2.5:2K	−84	477	43
41 biuret-free	2.5:2K	−35	203	
Percolates				
30-C-B	2.5:2K	−63	366	51
30-C-B biuret-free	2.5:2K	−33	189	
36-C-B	2.5:2K	−69	394	23
36-C-B biuret-free	2.5:2K	−16	91	
Combinations				
Perfusate 42		−38	407	
Extract 3	Double dose	−68		95
Biuret-free combination		−68	389	
Perfusate 43		−62		
Percolate 4-B		−80	1212	
Extract 8		−69		24
Biuret-free combination		−52	297	

* Double usual dose.

from the pancreas of the skate, since it gave "none of the color reactions for protein." Later Doctor Best (oral communication) has expressed the opinion that this product was not really protein-free. Abderhalden (1914) recently has stated that certain very effective insulin preparations did not give the ninhydrin reaction even when large amounts of the reagent were used, provided heating was not too prolonged. With longer heating the

reaction finally became positive in almost all cases. Starting with a preparation which gave a negative reaction with mild heating, when a little n/10 HCl was added and heating continued the reaction became positive. Since all α-amino acids give the reaction and it is only necessary to have one carboxyl and one α-amino group free Abderhalden's observation signifies only that an amino acid enters into the structure of some complex found in commercial insulin preparations. Boiling with n/10 HCl sets free the reacting groups but does not inactivate the insulin while boiling with weak alkali as we have often noted does inactivate and, according to Abderhalden, does not produce the ninhydrin reaction.

Abderhalden notes that insulin preparations give the picric acid reaction, indicating presence of a carbonyl group and boiling with acid or alkali does not materially change the reaction.

The evidence accumulates therefore that insulin *per se* is not a true protein in the sense of being composed of nothing but amino acids. Referring again to the insolubility of the preparation described above, it is worthy of note that Dudley (1924) has found the purest insulin obtained by him to be relatively insoluble in neutral water.

Organic analyses of the biuret-free insulin, owing to the uncertain yield and the extreme sensitiveness of the product to inactivation, are not yet complete.

SUMMARY

1. A method is described for obtaining biuret-free insulin from the perfusate of pig's pancreas. The yield is not large and the product is extremely sensitive to inactivation.

2. Insulin in this form is insoluble in neutral water and in weak acid but is readily soluble in dilute alkali and goes into solution at once in the body fluids. It gives none of the color reactions for proteins.

3. Attempts to prepare biuret-free insulin from ordinary aqueous-acid extracts of pancreas have not been successful although when combined with a perfusate the first filtrate from an acid extract will sometimes yield all of its insulin in this form.

4. From crude perfusates all of the insulin at times has been changed to the A-biuret form; from percolates as much as 51 per cent; from extracts by combination with a perfusate rarely more than 25 per cent.

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ROTATION AND ACCELERATION EXPERIMENTS, MAINLY ON FROGS

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The experiments recorded in this communication were undertaken after perusal of Prof. S. S. Maxwell's (1923) valuable and interesting monograph, "Labyrinth and Equilibrium." As his analysis of the physical factors involved in the problem of equilibrium seems to leave room for additional statement, a few preliminary remarks on general dynamical and physiological principles may not be out of place.

EXPOSITORY. *The gravitational field of force.* On our earth's surface any organism, animal or plant, is subject to a gravitational field of force, which acts uniformly and vertically. Suppose we refer any changes of position or of orientation of the organism to three fixed rectangular axes passing through an origin somewhere within its body, *i.e.*, to two axes XX′ and YY′, in the horizontal plane and intersecting at O, and to a third vertical axis OZ. Then it is plain that any rotation of the (otherwise stationary) organism about OZ is of no significance so far as gravity is concerned, for both during and after the rotation the various small elements of which its body is composed are subject to precisely the same gravitational field, acting, in reference to axes fixed by its own bodily configuration, in the same direction throughout. On the other hand any, even the slightest, rotation of the organism about either XX′ or YY′ alters the direction of the field of gravitational force relatively to axes specified in terms of its bodily configuration.

Instead of a living organism let us suppose that we are dealing with a four-legged table that stands on a level floor. Any displacement, linear or rotational, of the table, in which its four feet remain in contact with the floor, is of indifference so far as gravity is concerned; but if the floor itself is tilted and if friction is sufficient to keep the feet from slipping, then so soon as the vertical projection of the center of gravity falls outside the quadrilateral base of support formed by the feet, the table will topple over.

When a similar experiment is tried with a four-legged animal, *i.e.*, a tetrapod vertebrate, *and the movements of displacement are purposely made*

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very slow, simple rotation of the floor about a vertical axis is found to evoke no reaction whatsoever on the part of the animal. When however the floor is itself slowly tilted and rotation occurs about a horizontal axis, the living animal, unlike the inanimate table, reacts. If we describe its reactions in teleological terms, we may say that they are directed toward two ends: 1, to keep the plumb-line through the center of gravity well within the base of support, for which purpose the base of support, as by redisposition of the limbs, may actually be shifted; 2, to maintain the head, so far as may be, in its usual orientation relatively to the field of gravitational force.

These facts are illustrated in the accompanying four photographs (figs. 1 to 4). In figure 1 a frog is shown in a resting position on a level surface. In figure 2 the plane surface on which the animal rests has been tilted to an angle of some 50 degrees; the fore-limbs have been pushed forwards, thus serving to enlarge its base of support and to plumb the center of gravity within the extended base; simultaneously the fore part of the body is dorsi-flexed from the pelvic region forwards, the result being that the head is still held horizontal. In figure 3, the animal is shown tilted backwards; no new disposition of the limbs has as yet been made, but in the endeavor to keep the head so far as may be horizontal, the chin is brought in contact with the surface of support. Finally, in figure 4 is shown a frog which has been tilted laterally; the reaction whereby the center of gravity is plumbed within the base of support and the head kept as nearly horizontal as possible, is obvious.

The fixed or steady postures assumed as a result of completed rotation of the animal about a horizontal axis belong to the static group of reactions known as compensatory positions. These static reactions occur under a uniform field of force (in this case simple gravity).

Linear acceleration. By acceleration, in the simplest kinematical sense of the word, is meant rate of change of speed. If the acceleration is positive, as in a starting vehicle, the speed increases so long as the acceleration continues; if the acceleration is negative, as in a moving street-car to which the brakes are being applied, the speed diminishes. In such cases, where the motion is presumed to be along a straight line, the idea involved in acceleration is simple.

When the brakes are applied to a moving street-car, passengers standing on the floor, being urged forward by their inertia, find it necessary to lean away from the direction in which the car has hitherto been travelling. Suppose that the negative acceleration due to the brakes is temporarily *uniform:* on first application of the brakes, *i.e.*, when the acceleration is changing (and a skilled driver knows how to spread this change over some little time), compensatory movements are necessary; then, when the acceleration becomes uniform, a compensatory pose or fixed inclination of

the body is maintained; near the point of stopping the acceleration again changes and compensatory movements, leading to reversal of the temporary pose, are once more required.

From observations of this kind we are led to infer that the compensatory movements are associated with *change* of linear acceleration, whereas a compensatory position is associated with *uniform* linear acceleration; in connection with which statement we may note that the acceleration under consideration in these cases does not coincide in direction with gravity and that on an accelerated lift or elevator similar phenomena do not occur—yet see de Kleijn and Magnus (1921).

As the late P. G. Tait pointed out, it is of great convenience to extend the signification of acceleration, so as to cover cases where the applied force does not necessarily alter the *speed* of the moving body. Take the simple case of a bullet propelled horizontally from a rifle, and consider only the first short length of its course. So soon as the projectile, having within the barrel attained maximum speed, leaves the muzzle of the gun, it is subjected to the acceleration of gravity, which, acting vertically, *i.e.*, at right angles to the horizontal, does not alter the speed of the bullet, but begins to deflect it in a downward direction. Here the acceleration does not additively change the speed, but does alter the direction of movement. So soon as we reserve the term *velocity* for directed speed, and consider it as a vector quantity, the change of velocity implied in acceleration becomes itself a vector quantity and is added, not algebraically, but according to the rule for vector quantities.

This principle comes into consideration when a cyclist, in progress along a straight road, passes, let us say, from the shelter of a large wall or hoarding into the open, where a strong uniform wind is blowing at right angles to his path. Under the uniform acceleration due to the wind his speed is not changed (apart from the necessity of the first tack, when he has momentarily to head into the wind), nor is he himself, like the rifle

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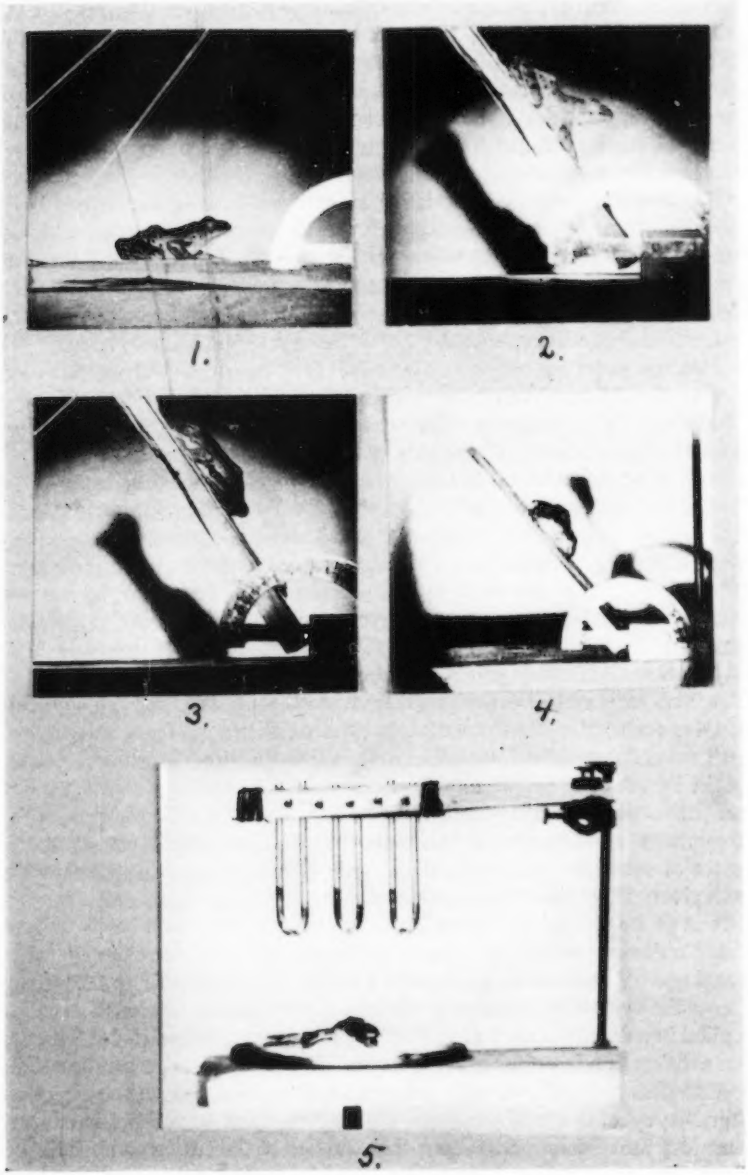
Fig. 1. A frog squatting normally on the horizontal plane.

Fig. 2. The frog tilted forwards through an angle of some 50 degrees. Note strong dorsi-flexion of back and forward extension of arms, the head being almost horizontal. That the body is driven well backwards between the two thighs—as much as in the frog of figure 1—can be seen by comparison of the tail-end in each.

Fig. 3. The frog tilted backwards. Note undoing of the pre-pelvic kink in the back and ventri-flexion of back, the head being so flexed that the snout touches the board. From the outline at the tail-end it can be seen that the body is held well upwards between the thighs.

Fig. 4. The frog tilted downwards to the left. By flexion of the right limbs, and downward and lateral extension of the left limbs, the head and body are kept, as far as may be, horizontal.

Fig. 5. Frog leaning to one side when subjected to centrifugal force. Note the strong lateral extension of the right limbs and the corresponding flexion on the left. Note also the difference in level of the mercury in the limbs of the U-tubes.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bullet, forced to deviate continuously from his rectilinear path. After the first compensatory movement due to exposure to a new acceleration, he leans toward the blast at an angle determined by the strength of the wind, and under this steady lean continues in his original straightforward direction. Here, as before, the compensatory movement is due to sudden *change* of acceleration, the steady lean or static pose to *uniform* acceleration in a direction other than that of gravity.

Whether the (uniform) acceleration be applied along the line of horizontal motion, as in our example of the street car passenger, or transversely to this direction, as in the case of the cyclist, we may say that the reacting individual comes under a uniform field of force, which is the resultant of that of gravity compounded with that of the particular horizontal force in question. Under this uniform field the reaction is a steadily maintained pose.

Centrifugal force. When we whirl a sling with a stone in it (for simplicity we will suppose the whirling to occur about vertical axes) we feel the tension of the cord, which constantly pulls the stone from its naturally straight path and forces it to move in a circle. When whirling is once well started and the hand is now held steady, *i.e.*, when the center of rotation is fixed, the force (or acceleration) applied along the string is wholly used up in diverting the stone from an otherwise rectilinear path. This case differs from that of the horizontally projected bullet in that the force in question (so-called "centrifugal force") does not, like gravity, remain constant in direction, but changes its direction so as always to be normal to the momentary course of the stone.

It is an easy matter experimentally to satisfy oneself that the acceleration (or force) thus instrumental in bending the stone from its straight path depends partly on the speed with which the stone is whirled, partly on the length of the string; and by simple kinematical considerations one can prove that the measure of the acceleration is V^2/R, where V is the momentary velocity and R the distance of the moving stone from the center of rotation. Consequently, when V is constant, the acceleration for a given R remains fixed in amount.

It is of no little interest to know how a rotated animal will behave under uniform centrifugal force. As a familiar case consider a cyclist coasting with uniform speed around a circular bend in a road. By reaction of the cycle wheels with the road an acceleration is now each moment applied in a direction normal to that of the momentary velocity. Though this acceleration is uniform in amount it constantly changes in direction; nevertheless the cyclist merely changes his pose relatively to the direction of gravity. At the beginning and at the end of the turn there is a compensatory movement, throughout the duration of the turn a compensatory pose. The same rule as before is observable, but with this addition, that

uniform change in the *direction* of acceleration, so long as it does not involve change in the *amount* of acceleration, is associated with no sequence or succession of compensatory movements.

Résumé. If, by way of summary, we now attempt to specify the nature of the reaction which one might expect from an animal subjected to any effective linear acceleration whose direction does not correspond with that of gravity, we should say that under constant acceleration the reaction will be the assumption of a compensatory position, whereas under varying acceleration it will involve the execution of a compensatory movement, or, if the acceleration goes on varying, perhaps of a succession of compensatory movements. The matter might equally be phrased in the following way: when $\frac{d^2v}{dt^2} = O$, the reaction is some compensatory position; when $\frac{d^2v}{dt^2} \neq O$, the reaction is compensatory movement. Before accepting these two generalisations as valid, one would naturally demand more evidence than has been adduced in their support, and it is our object here to make the evidence more complete. It should however be fairly clear to a physically minded reader that these two different forms of reaction are exactly the responses best adapted to meet the two separate physical conditions in question.

Experimental. *The threshold of angular acceleration.* As Maxwell's account seemed to make insufficient distinction between centrifugal force and tangential acceleration in cases of rotation, we were anxious to observe how rotated animals react to simple normal acceleration under constant tangential (or constant angular) velocity. Consequently we began by rotating pigeons, guinea pigs and frogs on a smoothly running horizontal turntable. To eliminate visual stimulation, the animals, illuminated from an artificial source which rotated with the table, and surrounded with a similarly rotating cylindrical screen, were observed during external darkness from a position screened against the source of light. Sometimes they were placed over the axis of rotation, sometimes at a distance from the axis. It was at once apparent that if the turntable is made to rotate with a very low angular acceleration, the animals make no turning movements either of eyes, of head or of body about a vertical axis, even though an eventual rapid rate of rotation is achieved. To elicit turning movements during rotation a certain minimal magnitude of angular acceleration is necessary, and the threshold is decidedly lower for the pigeon than for either the guinea pig or the frog.

These objective results with animals might be set alongside the subjective findings of Raymond Dodge (1923 a and b), who in experiments on blindfolded human beings subjected to rotation, determined that no sensation of turning is experienced until a certain threshold of angular acceleration is attained.

In order to keep below the threshhold and to avoid any sudden change in the gradient of angular velocity, a heavy flywheel was connected with the (very much lighter) turntable, and the whole system was driven by hand. With practice it became an easy matter to subject animals to quite a high speed of rotation without ever crossing the threshold of effective stimulation by angular acceleration. As our object was to eliminate stimulation by angular acceleration, and to concentrate attention upon the effect of uncomplicated normal acceleration, we devoted no particular effort to making measurements of the threshold value of angular acceleration. In general it may be said that for deeply cooled frogs an angular acceleration of less than 36 degrees per second per second does not excite.

The frog as a subject of experiment. The frog has on various occasions been used in rotation experiments, *e.g.*, by Schrader (1887), by Ewald (1892), by Laudenbach (1899), by Ach (1901), by Gruenberg (1907), and by Maxwell (1923), all of whom were concerned with the turning responses to angular acceleration.

For simple observation of responses of the intact or of the decerebrate animal, tetrapod vertebrates, which can be examined in air and under an uncompensated gravity field, are preferable to fishes, which must as a rule be examined in a medium of great density. The early observations were made chiefly on birds, but the responses of four-footed reptant animals can be more easily interpreted than those of birds. In the recent work of Magnus and co-workers (1914) guinea pigs, rabbits and cats have been freely used. As a subject for experiments on equilibrium the frog has its own particular qualifications. It readily bears decerebration. If inclined to be irritable and to spring off the table, it becomes most manageable on cooling. Its labyrinth too is easy of access, and, compared with that of a mammal, less intimately encompassed by bone; consequently, if ablation or nerve section operations on particular parts of the labyrinth are contemplated—see McNally and Tait (1925)—it allows of the very finest differential experiments.

In the dry air of a laboratory it is necessary to set the animal on a damp substratum (filter-paper was used) and to pour water frequently over its skin. Decerebration was practised on the anesthetised animal by a method in vogue in European laboratories, which consists of transverse, linear compression with specially constructed screw forceps (a "Harvard round-jawed clamp" will serve the purpose) of both cranium and brain at a level just between eyes and tympana. By this means the optic nerves are also crushed and visual stimulation eliminated. To avoid hemorrhage the clamp is left in position for twenty minutes, by which time the frog is beginning to recover from its anesthesia; compression is then undone in stages separated by intervals of a few minutes. Such cranium-compressed frogs are particularly favorable subjects for experiment; a day or two

after operation their reactions are beautifully sharp and clear; at the same time the animals are quiet and free from restlessness. So far as their equilibrium reactions go, normal and cranium-compressed frogs behave very similarly.

Some technical details. As it was necessary in experiments involving the magnitude V^2R to make considerable variation in the distance of the frog from the axis of rotation, we employed different turntable tops, using for all experiments with extreme size of the variable R planks of wood of adequate length mounted at their center. To avoid wind stimulation under high values of V, and also to prevent the animal from being flung off the table, we often covered the frog with a glass shade, which could be securely fixed in position.

To avoid the necessity of handling the animal after any volitional or accidental displacement, and to give it any requisite orientation in reference to the centre of rotation, the glass shade itself was mounted on a moveable circular plate (or orienting table), which in its turn was fixed upon the turntable. From the bottom of this plate and at its center a vertical peg projected; this fitted into each of a series of holes bored at different distances along the plank forming the turntable top. With the turntable brought to rest, it was thus possible without disturbance to the frog, to reorient it or even to alter its linear distance from the center of rotation.

The speed of rotation was signalled in the following way. A metal peg projecting horizontally from the vertical shaft of the turntable and revolving with it, made contact with a fixed piece of steel spring arranged tangentially to the circle traced by the free end of the peg. With each revolution electric contact thus occurred through an arc of some twelve or fifteen degrees, during which period of angular revolution a signal magnet interposed in the circuit made a mark on a more slowly moving drum the speed of which was separately recorded with a time-clock.

The only feasible method of recording compensatory positions of the frog during rotation was by means of instantaneous photographs, and a month or two was spent in attempts to improve the technique. The difficulties are these. To obtain a picture of adequate size the camera must be near; but the nearer the camera is to the animal the greater is the effect of angular displacement and the shorter must be the period of exposure. Short exposures in turn demand very powerful illumination, and it was only after repeated discomfiture involving the consumption of well over a hundred plates, that eventually we secured (through the kind help of the Associated Screen News of Canada, Ltd.) sufficient illumination to take passable pictures. While the photographs are sufficient to show the kind of response, we have, for reasons which will shortly appear, not sought to use them for measurement. Prof. L. V. King, of the Physics Department of McGill University, suggested to us an excellent method of

automatically exhibiting on the photographs the inclination of the resultant field of force at the moment. As is well known, the surface of a cylindrical mass of fluid, rotated about the vertical axis of the cylinder, forms a paraboloid of revolution. By hanging in the plane of the radius and above the frog a U-tube containing mercury, two closely adjoining points of the parabola are indicated, and a line joining these fixes both the tangent and the normal at that part of the curve.

Lateral lean under centrifugal force. In our very first experiments, before the nature of the compensatory reaction on rotation was clear to us, we had placed our animals with the longitudinal axis of their body in line with the center of rotation (sometimes their head was directed toward, sometimes away from the center). Provided the angular acceleration was kept low, they showed, contrary to our primitive expectation, no turning movements of their head or body. Reflection however suggested that the result might be due to the fact that the labyrinths or other relevant receptor organs on both sides were (apart from the *direction* of movement) symmetrically disposed with reference to a radius of the circle of rotation. We hastened therefore to place the animals in the line of the tangent to the circle of rotation, but even so, no turning movement of the head occurred. The frogs simply leaned over to one side, much as in the illustration of figure 4. When the turntable was gradually brought to rest again these leaning animals resumed their original or normal posture.

Our next supposition was that, placed thus tangentially, their body would swing in continuous movement so that its median, longitudinal, "vertical" plane would at every stage coincide with the resultant of the horizontal acceleration V^2/R and the vertical acceleration g. This rule obviously obtains in the case of a cyclist rounding a bend in a road. Experiment showed that the frog behaves in a different fashion from the cyclist. Suppose the animal has its right side toward the center of rotation (it matters not in which direction it travels), and suppose the value V^2/R is made continuously to rise in magnitude. At a certain stage the frog makes an abrupt shift, the right side of the body (more especially the fore part and head) suddenly dipping downwards—we have called this movement the "first shift." This new posture is now maintained for a time, until with a higher value of V^2/R, the frog again makes an abrupt movement ("second shift"), flexing its right arm and extending its left arm laterally, thus increasing the lean toward the right. Still later the left leg is suddenly extended laterally ("third shift") and now the animal is well over on its right side. Any subsequent attempt at readjustment results in the animal being thrown against the side of the bell-jar. Figure 5 is a photograph of a frog leaning to one side under rotation. The animal has made its third shift and is just about to be flung off the table.

These reactions are understandable when one considers that a frog is

not, like a cyclist, supported as it were on a knife-edge. Owing to its wide base of support it remains in equilibrium when a cyclist would long since have fallen. It is to be noted too that both the first and the second shift involve especially the skeleton of the fore part of the body; only at the third shift is a leg moved. This behavior is associated with the fact that in all postures of the animal on the level, the acetabula are carried low relatively to the glenoid fossae; the plantar surfaces too are more adapted for adhesion to the substratum than are the palmar.

The above is not a full description of the actual adjustment of the tangentially aligned animal to increase of V^2/R. Think of a man standing in a street car, his body fronting to the side of the car and his legs well astride. He may withstand acceleration of the car without actual shift of his feet, but the distribution of tension in his muscles undergoes marked change. So, less visible but nevertheless appreciable movements of the frog's body occur between the so-called "shifts." Advantage was however taken of the occurrence of these sharply marked shifts to rotate frogs at

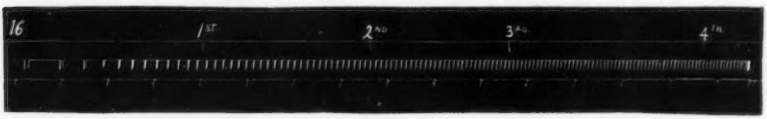

Fig. 6. Portion of record (considerably reduced in scale) to show the method of signalling the "shifts." Lower line—10-second intervals; middle line—revolutions of turn-table; upper line—point of occurrence of each shift at a distance of 16 inches from the center.

The tracing shows how even and regular the acceleration may be with a hand-driven drum properly weighted with a flywheel.

different distances from the center, and by simple observation, apart from photography, to signal with an electric key the moment of each shift on the drum on which was being recorded the momentary angular velocity. In this way a prospect seemed to offer of comparing the reactions of a given animal when subjected to equivalent horizontal (*i.e.*, normal) accelerations, though at different values of V.

The method of signalling is exhibited in figure 6, in which the lower line shows regular intervals of 10 seconds, the middle line single revolutions of the turntable and the upper line the (hand-signalled) moment of each successive shift of the frog. Knowing the distance of the frog from the center (in this particular case 16 inches), it is an easy matter from comparison of the lower and middle lines to reckon the value of V^2/R at the moment of each particular shift.

Varying the distance from the center of rotation. Many observations were made with the frog placed at 4, 8, 16, 24 and 32 inches respectively from the center. In each case care was taken to increase the angular velocity so

slowly as to excite no movements by simple tangential acceleration, the temptation to overstep the threshold being especially strong in the case of short distances. It was found that, whereas any given shift tends to occur at approximately the same value of V^2/R, the acceleration at which the particular movement occurs is not a fixed and invariable one. In the chart of figure 7, which represents the plotted average of a considerable number of observations, the accelerations responsible for the first and second shift and for throwing the frog bodily off the table are shown in terms of feet per second per second, for different values of R, in three successive tiers (for comparison the value of g, *viz.*, 32.2 feet per second,

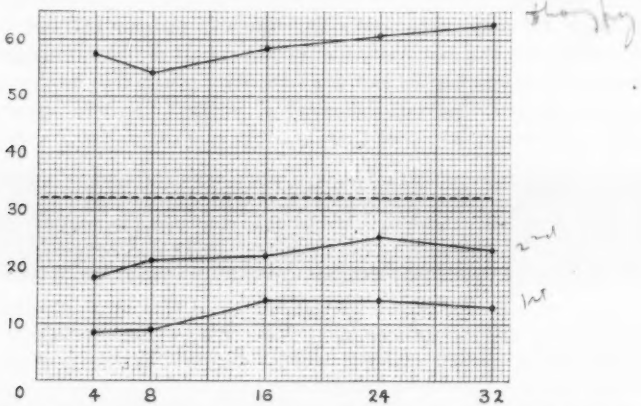

Fig. 7. Graph to show the acting centrifugal force at the moment of successive shifts of position of frogs rotated about a vertical axis and placed at different distances from the center.

Abscissa—distance from the center in inches.

Ordinate—centrifugal force in feet per second per second.

The dotted line, inserted for comparison, is the normal acceleration for gravity.

The graphs, in order from below, represent the first, second and final shifts.

per second is inserted in the same chart). Did each corresponding shift or displacement occur at a fixed value of V^2/R, each individual set of experimentally determined points would lie in a straight line.

One reason why a given shift or displacement does not invariably occur at the same value of V^2/R is simply that a frog, like a puppy-dog or like a human being, has a certain range of selection in the matter of posture, and does not always squat or adjust itself to lateral thrust in the same way. The chart however exhibits one definite peculiarity. At the lowest values of R the centrifugal acceleration sufficient to cause shift is less than at higher values of R. That this common feature of the three tracings should be a simple accident is unlikely. We are inclined to suppose—and exami-

nation of the records bears out the idea—that in the case of the lower values of R we had increased the angular velocity too rapidly and so had approached more closely to the threshold of stimulation by tangential acceleration; in other words that the peculiarity is a result of improper technique in the conduct of the experiment, and that the higher records are the more reliable.

One little point of difference observed between intact and cranium-compressed frogs was this. On being gradually brought to rest after rotation the intact frog always assumes its usual orientation relatively to the gravitational field; not so the cranium-compressed frog, which may remain in a leaning posture after rotation has ceased. Whether this difference is due to the want of vision in the cranium-compressed frog, we have not determined.

Rotation with radial orientation. If the frog, instead of being tangentially oriented, faces outwards from the center, it aiso reacts so as to resist the horizontal component of force. The head and anterior parts of the body are gradually raised, the arms are pushed forward so as to serve as buttresses, and the body as a whole is pulled backwards (center-wards) between the two flexed thighs. Before the animal slips and is flung against the bell-jar, its posture and orientation *with respect to the surface of contact* are exactly those of the animal illustrated in figure 2.

If on the other hand the frog faces the center, then, as the speed of rotation increases, it gradually bends its head till the lower jaw touches the table; the sharp pre-pelvic kink in its back is also undone—for its appearance at this stage *cf.* figure 3. Next the arms are extended laterally, and if the speed is further increased, the animal starts to creep towards the center, just as on a tilted board the imminence of slipping provokes a normal or decerebrate frog to climbing movements.

Such movements in which the animal creeps in obviously strained fashion towards the center of a rotating table or upwards on a highly inclined stationary surface, are worthy of the name "forced movements." The term is usually restricted to movements that result from lesions of the central nervous system—see Maxwell, chapter III—and there is no doubt a certain convenience in the convention. At the same time it is well to emphasize the minute similarity between a succession of movements of this labored and compulsory character due to extreme inclination of the acting field of force relatively to the plane of support, and that particular class of movements, following central nervous lesions, observation of which first suggested the epithet "forced" for their description—see McNally and Tait (1925).

The frog in the axis of rotation. If the frog is allowed to squat in the center of the table with the middle of its body over the central spindle, then, provided the angular acceleration remains low, it makes no detectable

movement even when the velocity reaches 1½ turns per second (the highest rate of rotation attained in our experiments). We have photographs of frogs spinning at this rate with the middle member of three U-tubes in dead center over the axis and with the arms of each of the two laterally arranged tubes respectively 1 inch and 2 inches from the center. The mercury in the lateral tubes shows a sharp difference of level, yet the frog squats just as it had been placed at the beginning of the experiment. If one reckons the labyrinths of the animal as being 1 inch from the center, the centrifugal acceleration at this distance is 7.4 feet per second per second, which number falls below the value for the "first shift" as shown in figure 7. When spun under these conditions the different parts of the animal's body are of course subjected to unequal and variously directed centrifugal forces, and it would be interesting, provided photographs could be taken, to see what ultimately happens as the speed of rotation is still further increased.

Effective rotation in a simple gravitational field. The reader may by this time have formed the conclusion, as we ourselves eventually came to see, that all these postures under the action of centrifugal force can be investigated just as precisely and with incomparably less trouble by placing the frog (a similar substratum being of course interposed) on a table pivoted about a horizontal axis and slowly rotating the animal under a simple gravitational field. On such a "tilt-table" the important factor in determining special posture is the angle of inclination, θ, of the table with the horizontal, or, what amounts to the same thing, the angle $\pi - \theta$, of the table with the vertical, *i.e.*, with the line of gravity.

In a rotation experiment (provided excitation by tangential acceleration is avoided) the effective field of acceleration at the moment has a magnitude and direction corresponding to the resultant of two accelerations at right angles to each other, *viz.*, g (vertical) and V^2/R (horizontal). The magnitude of this field is greater than gravity, but as we have seen from our illustration of the elevator, mere variation in *magnitude* of the field does not affect posture. The effective factor, corresponding to $\pi - \theta$ in the tilt-table experiment, is the angle between the plane of the rotating table and the existing field of acceleration, and this angle is evidently arc tan $g/(V^2/R)$, or arc tan gR/V^2. Comparison has amply substantiated this conclusion and it would only weary to give detailed figures in proof. Point for point (within the range of experimental error) exactly the same postural reactions can be elicited under equivalent angular conditions on the tilt-table as on the turntable, and here it is to be noted that equivalence is obtained only when the higher values of the chart in figure 7 are used for calculation.

The net conclusion is that under constant acceleration in a direction other than gravity the reaction is a compensatory pose, which pose can be

fully studied by the very simple means of using a table pivoted about a horizontal axis. These experiments may be compared with those of Kreidl (1892) on dogfish and (1893) on prawns.

The frog under simple linear acceleration. A jelly shape turned out of an inverted dish rests, let us say, on a plate on a polished horizontal table. If the plate is slid along the top of the table, the jelly, in virtue of its inertia, undergoes strain during acceleration. If a similar experiment is tried with a dead frog which has been propped into a squatting position (a substratum of moistened filter-paper is provided to prevent slip) the result is similar. If however a live frog is subjected in this way to horizontal acceleration, positive or negative, it reacts with the greatest promptitude and executes a movement of such a kind as not only to counteract the strain due to inertia but, if we may so speak, to overcounteract this strain. While its feet may preserve their original position on the plate, its body, relatively to its feet, actually executes an angular movement opposite to that which would occur in the jelly.

These facts might have escaped our notice, had we not observed that after special damage to the labyrinth the live frog under such circumstances may behave like the dead one. As observation shows that the movement coincides with change of acceleration, we have here another proof of the statement that any sensible change of acceleration whatsoever whose direction does not coincide with that of gravity tends to evoke compensatory movement.

In a succeeding paper—McNally and Tait (1925)—experiments on ablation of different parts of the labyrinth in their bearing upon compensatory positions and movements will be described.

SUMMARY

1. To change of simple linear acceleration in the line of gravity, as on an elevator, there is little or no reaction on the part of a normally posed animal.

2. To change of linear acceleration in a direction other than gravity the animal reacts by some compensatory movement. To constant linear acceleration in a direction other than gravity, it reacts by assuming a compensatory position.

3. Animals rotated on a horizontal turntable, make no turning movements of eyes, of head, or of body, about a vertical axis, provided the speed of rotation is raised very gradually. A certain threshold of angular acceleration is necessary to evoke such turning movements.

4. Under constant centrifugal force a frog adopts a compensatory position, which exactly corresponds to the compensatory position assumed when the frog has been tilted about a horizontal axis.

5. The effective factor in determining the pose under uniform centrifugal force (rotation being about a vertical axis) is the magnitude of the angle between the horizontal plane and the direction of the resultant force due to the two components of gravity and of the momentarily acting centrifugal force. Similarly the effective factor in determining the pose of a frog resting on a table which has been tilted about a horizontal axis is the angle between the table top and the plumb-line of gravity.

6. Suppose we consider any acceleration whatsoever whose direction varies from that of gravity, it is a general law that change in magnitude of this acceleration evokes compensatory movement, whereas a compensatory position is associated with constancy or uniformity of this acceleration.

7. A frog, facing the center of rotation on a rapidly revolving turn-table or directed upwards on a highly inclined stationary surface, executes forced movements of obviously labored and straining character.

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ABLATION EXPERIMENTS ON THE LABYRINTH OF THE FROG

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Though total ablations of the labyrinth have frequently been carried out in the frog, this animal has not hitherto been used for differential ablations or experiments on individual parts of the labyrinth. In the frog that organ is considerably smaller than in a pigeon or in a dogfish, and the operations are correspondingly more difficult. At the same time it was an obvious step, after our preliminary work on rotation and acceleration—Tait and McNally (1925)—to endeavor, by nerve section or otherwise, to throw individual parts of the frog's labyrinth out of action, in order to determine the corresponding disabilities of the animal. With practice it proved possible in a minority of the cases operated on to effect perfectly clean and sharp-cut local ablations (these were all carried out by W. J. McN.). In some preliminary experiments the American bullfrog, *Rana catesbiana*, was used, but in spite of the larger size of its labyrinth it proved easier, because of their relatively softer cranial bones, to operate on *Rana sylvatica* and on *Rana palustris*, and the experiments here recorded refer exclusively to these two species.

Operative technique. Ether anesthesia was used for all operations. A sponge is moistened with ether and put into a covered bowl with the frog to be anesthetised. So soon as the frog lies on its back without turning over it is sufficiently under the anesthetic to allow of operation. Induction of anesthesia takes 3 or 4 minutes and the effect lasts 2 or 3 hours. All operations were carried out under the dissecting microscope, to which a special little electric lamp was fitted for illumination. The instruments included dental drills and fine scoops for bone, eye-needle knives and Ewald's scissors for nerve section, and a specially fitted fine electric cautery with platinum wires of different form and thickness.

In order functionally to eliminate a semicircular canal two methods were used, 1, section of the nerve going to the ampulla, 2, cauterization of the ampulla over the area of insertion of the nerve. The first method is the more difficult, because in sectioning the nerve any pulling is apt to damage

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other structures in the labyrinth. This is particularly true in the case of the nerves to the anterior vertical and to the horizontal ampullae owing to the close proximity of the utricle. However, with care and the use of a very sharp eye-needle knife, the operation even on these nerves was successful in some cases. The cautery method is more easy of application if the temperature of the cautery is well controlled, a dull red heat being the optimum.

For sectioning the nerves to the anterior vertical or to the horizontal ampullae a cautery incision is made in the roof of the mouth just at the posterior edge of the orbit. With a small drill an opening is made into the labyrinth through the anterior wall of the pro-otic bone immediately over the ampulla to be operated upon. When the nerves to the ampulla are identified, the one desired is sectioned by using the eye-needle knife. Bleeding from the soft tissues is usually prevented by using the cautery for the incision. For cauterization of the anterior vertical or of the horizontal ampullae a cautery incision is made on the outer surface of the head between the eye and the ear. The pro-otic bone is opened just on the antero-superior edge over the desired ampulla. When the connection of the nerve with the ampulla is identified, the ampulla is gently cauterised, care being taken not to injure the adjacent ampulla.

For the posterior vertical ampullae a cautery incision is made in the roof of the mouth behind the floor of the labyrinth. The opening into the labyrinth is made external to and below the opening of the perilymphatic sac. The underlying posterior vertical ampulla is destroyed with the cautery. Here the danger of injuring other receptors is not so great as when one operates on the anterior ampullae.

It is to be understood that in operations on the semicircular canals the object was not in every case to abolish the function of just one ampulla. Two or more ampullae, in a variety of combinations, may be physiologically obliterated, and the study of these combined lesions serves as a useful check upon ablations of a single ampulla.

The method of operating on the saccular otolith and macula is to make a cautery incision in the roof of the mouth over the floor of the labyrinth. A small opening is made through the cartilage of the labyrinth and by the use of Ewald's scissors the nerve to the saccular macula is sectioned. In a few animals the operation was limited to destroying the pillars and supporting threads of the macula or to rupturing the otolith capsule and washing out its contents.

Owing to their inaccessibility it proved impracticable to operate on the utricular otolith and macula.

Testing apparatus. To test the reactions to rotation about a vertical axis the turn-table described in Tait and McNally (1925) was used. To test gravity responses on an inclined plane, or kinetic reactions on rapid

rotation about a horizontal axis, the tilt-table illustrated in figure 1 was used; this is provided with a handle for quick rotation through a limited arc. To test reactions to simple linear acceleration a smooth board sliding on a smooth horizontal table was employed, the board being moved by hand. In every case wet filter-paper was interposed between the animal and the surface of the testing apparatus.

KINETIC REACTIONS OF A NORMAL FROG. It is evident that in any kinetic test two differently directed accelerations must inevitably occur, and either may act as an adequate stimulus. There is 1, the positive acceleration on starting, and 2, the negative acceleration on slowing down. In experiments on linear acceleration, for example, as carried out by us, these are brought successively into play within a very short interval of time; so too in rotation about a horizontal axis. Consequently attentive observation and some judgment are necessary in order to discriminate between reactions to positive and reactions to negative acceleration. With experience however there is less risk of confusion.

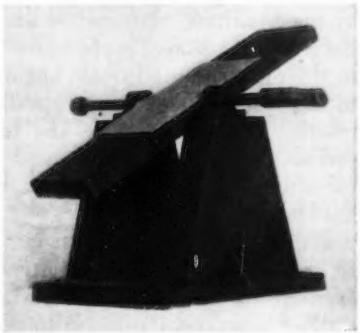

Fig. 1. The tilt-table, capable of rotation in either direction, and provided with a handle for turning.

It will simplify the exposition if we here comment very briefly on the reactions of the normal frog to 1, linear acceleration, 2, rapid rotation about a vertical axis, and 3, rapid rotation about a horizontal axis. Then, in describing the reactions of the operated frogs, it will be sufficient to refer mainly to the exceptional features presented.

The reactions to acceleration of any frog, whether normal or with damage to the labyrinth, are more constant if means are taken to eliminate visual stimulation. This can be done either by cranial compression as described in our previous paper, or by surrounding the animal with a cylinder which moves with the table.

Linear acceleration. The limb-supported body of a healthy frog suddenly subjected to simple linear acceleration exhibits a momentary lag due to inertia. This is well seen if the animal is arranged to squat with its longitudinal axis at right angles to the direction of acceleration, but also if it is arranged in the line of the acceleration; just for an instant it is seen to sway in the direction opposite to that of the temporary acceleration, positive or negative. The striking feature about the animal however, to which we referred in our previous paper, is that the lag due to inertia is slight and moreover is instantly corrected, the frog executing with great

promptness a movement in the contrary direction. In proportion as the animal is weak or out of sorts, so is the alertness of its neuro-muscular system depressed and it yields to inertia without displaying this physiological counter-reaction.

In actual practical testing it is easier to make the observations at the *end* of a linear translational movement, *i.e.*, when negative acceleration is acting, than at the commencement of or during the linear movement. In subsequent descriptions of operated frogs the reactions to negative linear acceleration are given by preference. As the orientation of the animal can always be reversed, it is plain that all necessary information can be obtained from observations made exclusively during negative acceleration.

Rotation about a vertical axis. The responses to angular acceleration when the frog is rotated on an ordinary turn-table have been described by many writers. No matter where or in what orientation relatively to the center the animal is placed on the table, on commencement of rotation to the right under adequate angular acceleration, the frog turns its head to the left, and may start to walk round and round to the left. When rotation is made to cease and the direction of angular acceleration is reversed, the frog turns its head to the right and may execute several turns to the right, even continuing to do so for a little after the table has stopped.

Rotation about a horizontal axis. On the tilt-table the frog may of course sit in various orientations relatively to the axis of rotation. In routine examination three main orientations are important, *viz.*, A, with longitudinal body axis transverse to the axis of rotation, the frog being tilted either forward or backward; B, with longitudinal body axis parallel to the axis of rotation, the frog being tilted to one or other side; C, an intermediate orientation between A and B, the animal being so placed that the plane of a right and left pair of vertical semicircular canals is transverse to the axis of rotation. When the frog is posed at the beginning of an experiment the tilt-table is horizontal; from this position rotation can be carried out through an arc of some fifty degrees. Once the table is tilted however, the available arc of rotation is doubled. In these rotation experiments, as contrasted with experiments on linear acceleration, simple inertia plays little or no part. If the animal is near the axis of rotation the actual translational velocity and consequent momentum are never high. Thus the body of the animal shows no particular lag.

On rotation forward, in orientation A, the head is promptly raised, and both arms are tonically extended at the elbow and rotated about their long axis (elbows inwards) at the shoulder so as to stand parallel. When the rotation stops, this position, which is similar to that adopted after slow tilting forward, is maintained. On rotation backward, there is prompt

lowering of the head and undoing of the pre-pelvic kink, with extension and outward throwing of the arms, which are also rotated about their long axis at the shoulder (pre-axial border downwards) as when a man makes a stroke in swimming; if the rotation is through a wide angle, the legs are also extended laterally and posteriorly. On cessation of the rotation, this position, which is similar to that adopted on slow backward tilting, is maintained.

On rotation to the right, in orientation B—here the rotation must not be so sudden as to cause the animal to lose its footing—the head and anterior part of the body are promptly tilted to the left by lateral extension of the right arm and simultaneous flexion of the left arm, with (later) a lateral extension of the right leg. When rotation stops there is very little lateral swaying of the body, and the position, which is similar to that assumed in response to slow tilting to the right, is maintained. An analogous response occurs on rotation to the left. The compensatory movements in these cases are decidedly prompt and show every sign of vigorous innervation.

Suppose the frog to be placed in orientation C in such a way that a line from the left shoulder to the right hip is parallel to the axis of rotation, and suppose that the rotary movement is forward and to the right. The right arm is immediately extended and the left is partially flexed, so that the head is lifted on the right side; the right leg is extended laterally and moved forward. This position is maintained when the rotation stops. When the rotation is backwards and to the left, the head is lowered, and both arms (chiefly the left) are extended laterally; the legs are extended, the left being carried well back and outwards. This position is maintained when rotation stops. These responses are always prompt and unaccompanied by swaying; in each case the result is to maintain the head and eyes nearly horizontal.

In contrast to the case of linear acceleration, when observations are most easily made at the finish of the translatory movement, it is more satisfactory to note the reactions to rotation on the tilt-table at the commencement rather than at the finish of the rotation. This holds particularly true in the case of operated animals, which are apt by undue movement to be upset towards the end of angular displacement.

By way of review we would again emphasize the fact that these various kinetic reactions of normal animals are at each stage accurately adjusted, so that when movement of the table stops the animal is already in a posture suited to the circumstances of the moment, which posture it now maintains without the necessity of further adjustment. As we shall see, injury to the semicircular canals interferes with the prompt, and therefore continuous, execution of these adjustments.

FUNCTIONAL ELIMINATION OF AMPULLAE OF SEMICIRCULAR CANALS. *General features.* In the course of a large number of experiments certain points became quite plain. No lesion of semicircular canals causes a forced position. The only stimulus to the ampullae is an acceleration, and the effector response resulting from their stimulation is a compensatory movement. The canals are not concerned with the static postures assumed in relation to the field of gravity, or to centrifugal force.

All six ampullae. After total ablation of all six ampullae the frog squats naturally and, so long as it stays at rest, shows no abnormality. The responses to slow tilting about the transverse, longitudinal and oblique axes of the body are normal; so is the response to centrifugal force. The righting reaction is quite prompt.

The frog itself is loath to jump and in spontaneous movement creeps cautiously. If forced to jump, it "takes off" without direction and lands in any position. In swimming there is marked swaying from side to side and no uniform direction is maintained; the legs however are kicked out simultaneously.

Subjected to positive linear acceleration in the forward direction, the body slides backwards between the flexed thighs; with ensuing negative acceleration the body now slides so far forwards that the nose touches the table. When linear acceleration ceases the normal attitude is again assumed. Subjected to positive linear acceleration to the right, the body sways markedly to the left, and *vice versa;* when the lateral acceleration ceases the animal recovers its normal position. Apart from this ultimate recovery of the normal position, the movements are to some extent like those of a dead or dying frog which has been propped up in a squatting position; only, the operated frog, like a man with intention tremor, appears itself to impel its body in the wrong direction. It seems as if the swaying movements are not wholly due to inertia.

To angular acceleration about a vertical axis there is no turning response of head or body. On forward rotation on the tilt-table, the body lurches forward with head down; then, as the rotation ceases, the head is raised, the arms are extended and the body pushed back, and this position is maintained on the stationary inclined plane. On rotation backward from the horizontal, the body lurches backward, the head is thrown up and the hands may for a moment lose contact with the table. Then as rotation stops, the head is dropped to the board, the arms being extended laterally and the legs laterally and posteriorly, which position is maintained on the stationary inclined plane. On rotation to the left the body at first slews markedly to the left; then, as the rotation ceases, the left arm and leg are laterally extended, the right arm is flexed and the left side of the body is raised, which position is maintained on the stationary inclined plane.

All responses of the animal on the rapidly rotated tilt-table are mala-

droit, and with any undue angular velocity or displacement the frog is apt to become unbalanced and to fall off. A dead or debilitated frog does not react in quite the same way, being less easy to dislodge than the frog deprived of all ampullae. The backward propulsion of the body on backward rotation would suggest that muscle and tendon receptors of the extensors of the fore-limbs are stimulated, and owing to the uncontrolled reflexes thus initiated the forepart of the body is actively lifted and flung backwards. Of this latter supposition however we have no proof.

As the complete operation is a severe and extensive one, a control animal was subjected to all the external incisions for approach to the ampullae, but without opening the labyrinths. Two hours after these operations its responses were those of a normal frog. The conclusion is that the specific nature of the ampullary operation, and not its severity or extent, causes the absence of the normal swift and accurate adjustments to acceleration.

Ampulla of left horizontal canal. Posture normal; righting reaction prompt; responses to gravity and to centrifugal force normal. The animal appears to swim normally, no disability being detected by us. In jumping it shows a slight tendency to turn to the left.

We are unable to specify with precision the reactions to linear acceleration on the sliding board. In one case it seemed that to negatively accelerated forward movement there was a slight slewing of the body forward and to the left, and to similar negative acceleration with the left side in advance a slewing of the body to the left. In three other animals we failed to repeat these observations.

On the turn-table there is no response during positive angular acceleration to the left; as rotation stops the animal turns its head to the left. On positive angular acceleration to the right the turning reaction is normal, but on cessation of the rotation there is no turning response. If the ampulla of the right horizontal canal is obliterated in an otherwise intact animal, these reactions are reversed. Hence it is plain, as Crum Brown (1874) suggested, that a horizontal canal is stimulated by rotation in its own plane in one direction but not in the other. The one direction for effective stimulation is, in this particular case, that in which the narrow part of the canal travels first and the ampulla brings up the rear. On the tilt-table all reactions are normal.

Ampulla of left anterior vertical canal. Squatting posture normal; righting reaction prompt. The animal appears to swim normally, kicking the two legs simultaneously. Response to gravity, to centrifugal force, and to angular acceleration on the turn-table, normal.

In crawling and especially in jumping there is, if anything, a tendency to turn to the left. When, by the sliding board, the frog is pulled backwards, *i.e.*, when it is subjected to negative acceleration after travelling

head first, the head is depressed, turns slightly to the left, and is also tilted downwards on the left. When the frog is pulled forward, *i.e.*, when it is subjected to negative acceleration after travelling tail-end first, the reaction is normal and symmetrical. When the frog is by the board pulled to the right, the head is kept down and turns laterally to the left, and on stoppage of the acceleration comes slowly back to the mid line; the left fore-limb is kept flexed and does not move in this reaction. When the frog is pulled to the left, the reaction is more normal, *i.e.*, the head moves up and to the right through a much wider arc, the right fore-limb being extended and used as a buttress; on stoppage of the acceleration the head now swings back past the mid line, after which it returns to its ordinary position in line with the body. When the frog, obliquely oriented, is pulled back in the plane of the damaged canal the head is depressed and tilted downwards on the left; the right arm may move, but not the left. When the frog is pulled forward in the plane of the damaged canal, it shows no particular abnormal reaction. When the frog, obliquely oriented, is pulled either backward or forward in the plane of the undamaged anterior vertical canal, the reactions are normal. These experiments establish a functional correlation between the left anterior vertical canal and the left fore-limb; one infers that adequate stimulation of the intact canal, by activating the left arm, lifts the shoulder-girdle and head on that side, at the same time thrusting the body towards the right.

When on the tilt-table the frog is rotated forward, the body is raised by the right arm, but the head is tilted downwards on the left side owing to inadequate extension of the left arm; this occurs during the forward acceleration, and on stoppage of the rotation a normal pose for that inclination is assumed. When the animal is rotated backwards, there is no abnormal reaction. On rotation to the right the response is normal. On rotation of the frog to the left the head and anterior part of the body, owing to want of thrust from the left arm, slew more to the left than in a normal frog. When the frog, obliquely oriented, is rotated forward in the plane of the damaged canal, the head and anterior part of the body slew markedly forward and to the left. On rotation backward, in a similar orientation with respect to the axis of rotation, there is no abnormal response, nor when the animal is rotated with the plane of the damaged canal parallel to the axis of rotation.

These rotation experiments, like those with linear acceleration, go further to establish a functional correlation between the left anterior vertical canal and the left fore-limb of the kind already indicated. A striking feature however is this. In order that positive linear acceleration in the horizontal plane should act as a stimulus, the canal must be so oriented that the ampulla brings up the rear and the narrow part of the tube is in advance. In order that positive angular acceleration in the plane of the

vertical canal should stimulate, this direction is reversed. It would seem at first sight as if the mechanism of stimulation by linear acceleration is different from that by rotation. Later in our Discussion on Semicircular Canals we shall consider this point.

Ampulla of left posterior vertical canal. Squatting posture normal; righting reaction prompt. The animal appears to swim normally, and kicks the hind-limbs simultaneously. Response to gravity, to centrifugal force, and to angular acceleration on the turn-table, normal.

In crawling and particularly in jumping, there is a tendency to turn to the left. When the frog is pulled backwards by the sliding board, its response is normal. When it is pulled forward, it shows a striking abnormal response, the body being lifted in front and, as it were, flung back towards the left by inertia; on stoppage of the linear movement, provided the animal has not been overbalanced, the normal pose is resumed. This characteristic upward and backward fling of the body towards the left is more marked the nearer the direction of acceleration approaches the plane of the injured canal; the tendency to overbalance is also greater when the frog, to begin with, is sitting up rather than squatting flat, and is due mainly to inadequate action of the left hind-limb. When a normal frog is pulled forward, both hind-limbs, without however shifting their position on the table, immediately react and thrust the body forward; in the absence of the left posterior canal the left leg does not react to these conditions, whereas the right does; hence the peculiar throw of the body towards the left. When the animal is pulled by the board, to the left, the response is normal; when it is pulled to the right, the body is flung for a moment backwards and to the left.

When, on the tilt-table, the frog is rotated forward, no particular abnormality is observed. On similar rotation backward a peculiar and characteristic reaction occurs. A normal frog, to save itself from falling, extends both arms and both legs. The operated frog simultaneously extends its right arm laterally and its right leg laterally and posteriorly, but does not move its left arm or left leg; as a consequence the body is tilted toward the left and sags backward towards the left. On stoppage of the rotation a normal pose for that inclination is assumed, that is to say, the arm and leg on the left side are also extended, the head is lowered, etc. On rotation to the right the response is normal. On rotation to the left the body, from want of innervation especially of the left hind-limb, slews obliquely backwards and to the left. When the frog, obliquely oriented, is rotated backward in the plane of the damaged canal, the body is lifted backwards and to the left; as under these conditions balance depends principally upon the non-responsive left hind-limb, the frog is very easily upset, toppling backwards and to the left. When the animal, with similar orientation, is rotated forward, there is no abnormal response, nor yet when it is rotated with the plane of the damaged canal parallel to the axis of rotation.

By these experiments, just as by the experiments on the anterior vertical canal, two outstanding facts are brought into relief. First, each vertical canal stands in close functional association with a particular limb, the left anterior with the left fore-limb, and the left posterior with the left hind-limb. Secondly, the mechanism of stimulation of the canal by positive angular would seem to differ from that by positive linear acceleration; for the former the ampulla must go in advance, for the latter the ampulla must bring up the rear—see under Discussion on Semicircular Canals.

Ampullae of both anterior vertical canals. To carry out an uncomplicated ablation of the ampullae of the two anterior vertical canals is an exceedingly difficult operation, and we have in no case succeeded. The difficulty is to avoid some damage to a horizontal canal. At the same time it is, we think, justifiable to deduce from a number of experiments, each with a slightly different degree of involvement of horizontal canals, including some with an intact horizontal canal on one side, what would be the result of an uncomplicated ablation of the two anterior vertical ampullae.

In jumping, the animal gets well off the ground, but lands with both arms spread outwards, so that the sternum strikes the ground at the same time as the hands. In this position the animal may slide forwards on its belly before gathering itself together for another jump. When released from the hand under water it tends to swim downwards to the bottom and along the bottom, both hind-limbs being kicked simultaneously.

When, by the sliding board, the frog is pulled backwards, its body slides forward, the head dips down, and the arms, with actual displacement of the hands on the board, are spread outwards; only when the movement stops does the animal resume its normal position. When the frog is pulled forward, the response is normal. When the animal is pulled to the right, the head sways laterally to the left and is tilted downwards on the left; on stoppage of the acceleration it comes slowly back to the mid line. When the frog is pulled to the left, the response is similar, but in the opposite direction.

When, on the tilt-table, the frog is rotated forward, the body slides forward, the head is lowered; and the arms tend to spread out; only on stoppage of the acceleration is the normal pose for that inclination assumed. On rotation backward the response is normal. On rotation to the left or to the right the response is similar to that of an animal with the ampulla of only one side destroyed when rotated to the operated side. Similarly, on rotation about an oblique axis either forward and to the right or forward and to the left, the response is the same as that of an animal with the canal of only one side destroyed when rotated forward to the operated side. On rotation backward about either oblique axis the response is normal.

On the turn-table the reactions are normal.

The fact should be particularly noted that this animal, whether pulled backwards on the sliding board or tilted forward on the tilt-table, makes a definite movement with its arms in the opposite sense to that of a frog with intact anterior vertical ampullae.

Ampullae of both posterior vertical canals. In jumping, the animal puts less innervation into its legs than into its arms, so that it shoots upwards rather than forward, and as often as not turns a somersault backwards. No matter in what direction it is released under water, its swimming efforts, in which both legs are kicked simultaneously, drive it to the surface, where, with body vertical, it urges itself upwards as much as forwards.

When it is pulled forwards by the sliding board, its body is thrown upwards and backwards; there is distinct sliding of the body backwards between the flexed thighs; the hind-limbs do not move, as they immediately do in a normal frog. When the frog is pulled backwards its response is normal.

When, on the tilt-table, it is rotated backwards, the fore-part of its body is thrown upwards and backwards. It can be seen that the fore-limbs, by extension and inward rotation of the elbows, are suddenly straightened (in a normal frog under similar conditions they are thrown out laterally). Meantime the hind-limbs remain motionless and limp, and the body sags backwards between the flexed thighs. The frog is with great ease thrown backwards off the tilt-table.

Here the peculiar fact should be noted that on backward rotation the frog makes a definite movement with its arms in the opposite sense to that of a frog with intact posterior vertical ampullae.

Ampullae of left anterior vertical and of left horizontal canal. Whereas a frog with only one of these ampullae obliterated shows a slight tendency to turn to the left, an animal with the double operation shows a decided tendency to turn to the left in crawling, in jumping, or in swimming.

When, by the sliding board, the frog is pulled backward, the head is depressed, turns slightly to the left side, and is also tilted downward on the left. When the animal is pulled forward the response is normal, except for a possible slight raising of the head, more on the right side than on the left. When the frog is pulled to the right, the head sways strongly to the left, the left side of the head being markedly depressed, so as almost to touch the table; on stoppage of the acceleration it comes slowly back to the mid-line. When the frog is pulled to the left its response is normal.

On the tilt-table the reactions are similar to those described for an animal with only the left anterior vertical ampulla destroyed. On the turn-table the responses are like those of an animal with only the left horizontal ampulla destroyed.

Ampullae of both anterior vertical and of both horizontal canals. The behavior of the animal on jumping is not unlike that of a frog in which the

ampullae of the two anterior vertical canals have been obliterated. On "taking off" however the frog sways more from side to side. This behavior in turn is like that of an animal with all six ampullae obliterated. There are however two points of difference: a frog with the complete operation sways backwards and forwards as well as from side to side, like a table on insecure legs; a frog with the complete operation is also less readily induced to jump. In swimming, the animal with the four ampullae destroyed tends to go to the bottom but sways markedly from side to side.

Its reactions on the sliding board and on the tilt-table are like those of an animal with the ampullae of both anterior vertical canals destroyed. At the same time it shows absence of any reaction to angular acceleration on the turn-table.

Ampullae of three semicircular canals of the left side. Squatting posture normal; gravity reactions and reactions to centrifugal force also normal. In jumping, the animal has a distinct tendency to turn to the operated side, and on landing momentarily leans downwards to the operated side. In swimming it turns to the left; though the two legs kick simultaneously the left leg is extended laterally more than the right.

When, by the sliding board, the frog is pulled backwards, the head is depressed, turns slightly to the left side, and is also tilted downward on the left. When the frog is pulled forward, the body is lifted in front and by inertia flung back towards the left; on stoppage of the acceleration the normal pose is in each case resumed. When the frog is pulled to the right, the body is flung markedly to the left, the left side being depressed; on stoppage of the acceleration the body returns slowly to the mid-line. When the animal is pulled to the left, the response is normal.

To angular acceleration on the turn-table the responses are those of a frog with only the left horizontal ampulla destroyed.

When, on the tilt-table, the animal is rotated forward, the response is that of a frog with only the left anterior vertical ampulla destroyed. On rotation backward the response is that of an animal with only the left posterior ampulla destroyed. On rotation to the right the response is normal. On rotation to the left there is a marked slewing of the body to the left, with depression of the left side, so that overbalancing is easy; on stoppage of the acceleration a normal pose for that inclination is assumed. When the frog, obliquely oriented, is rotated forward and to the left, the response is that of an animal with only the anterior vertical ampulla destroyed. On rotation backward and to the right with the same oblique orientation as in last experiment, the response is abnormal to this extent that the head is slightly raised on the left side with a slight lateral tilt to the right. When the frog, obliquely oriented, is rotated forward and to the right, the response is normal. When, preserving the same orientation with respect to the axis, it is rotated backward and to the left, the response

is similar to that of an animal with only the left posterior vertical ampulla destroyed.

Discussion on semicircular canals. By the foregoing experiments certain features, general and special, are rendered plain. To the general features we referred at the beginning of last section. The more obvious special features are these: 1, Whereas effective stimulation of one horizontal canal can throw the musculature on both sides of the body into play, each of the four vertical canals is concerned only with the musculature of its own side. 2, There is a particularly close relation between an anterior vertical canal and the forelimb of the same side; there is a similar intimate relation between a posterior vertical canal and the hind-limb of its own side. 3, The effective stimulus to a horizontal canal is a positive acceleration, angular rather than linear, in such a direction that the narrow end of the tube is made to move first, the ampulla bringing up the rear; there is no evidence of stimulation when the ampullary end is made to move first. 4, To positive linear horizontal acceleration applied in any particular direction, the vertical canals react in the same way as when the animal, from appropriate sudden inclination of the horizontal plane, tends to fall in the opposite direction.

The geometrical arrangement of the canals. Crum Brown pointed out that the arrangement of the six canals is such that, when they are paired off in planes, the ampullae of any pair in the same plane are at opposite ends, and that, once the plane of the horizontal canals is fixed, no other possible disposition of the planes of a bilateral organ can fulfil this condition. His inference was that rotation of a given plane in one angular direction stimulates the ampulla at one end, and rotation in the opposite direction stimulates the ampulla at the other end. On the validity of this ingenious induction, doubt has been cast by experimenters. Our experiments indicate that the arrangement of the four vertical canals meets another condition, being in fact peculiarly adapted to the four-limbed vertebrate type of organization. The direction of these four canals with their terminal ampullae is appropriately and equably adjusted with reference to the four "quarters" of the animal's body, each with its own limb. This correlation is perhaps all the more plain in the case of a short and broad tetrapod animal like the frog, in which the limbs are so obviously placed at the four corners of the body.

The adequate stimulus to the canals. While many observers are agreed that the left horizontal canal is stimulated only by positive angular acceleration to the left and by negative acceleration to the right, and that the right horizontal canal is stimulated by the contrary accelerations in each case, the matter is not quite so clear with regard to the direction of angular acceleration for stimulation of a given vertical canal. Ewald (1892) clearly showed that a vertical canal is most readily stimulated when

the rotational movement is such as to carry the ampullary end of the canal in advance, the rule being here reversed as compared with that for the horizontal canals. This conclusion, abundantly confirmed by our experiments, has never been contravened by other observers.

There is an opinion which originated with Ewald, that a vertical canal is likewise stimulated, but to a less degree, by rotation in the opposite direction; that, in other words, its ampulla acts in a differential fashion and gives origin to two sets of afferent nerve fibres, a major set inducing one response, and a minor set helping out the major set of the opposite ampulla. Our experiments on bilateral ablation at one time of anterior, at another time of posterior vertical ampullae, give support to this idea. In each case anomalous movements of the forelimbs occur, which seem to be explicable only on the hypothesis that the opposite (intact) vertical canals are stimulated by a movement in which the narrow end of the tube moves first. That these unusual arm movements are not due to simple tendon reflexes is shown by the fact that they are absent in a frog from which all six ampullae have been removed. If the vertical canals contain a differential mechanism, our previous statement that each of the four is concerned only with the musculature of its own side of the body, is only relatively and not absolutely true.

In connection with the vertical canals reference may be made to the subject of horizontal linear acceleration as an adequate stimulus. It is quite plain that on the sliding board frogs with lesions of the vertical canals show reactions similar to those exhibited when the same animals are so tilted that they tend to fall in a direction opposed to that of the linear acceleration producing a particular abnormal response. It is however to be noted that, by means of the sliding board, the horizontal force is applied, not directly to the body or head, but indirectly through the legs; the frogs "have their feet pulled from under them." An inevitable result must be that the superimposed body with attached head executes a rotational movement similar to that which occurs when the animal, by inclination of the horizontal substratum, is directly rotated towards the opposite side. Thus is resolved the apparent and consistent anomaly of our previous section whereby linear acceleration and rotation seemed to act in opposite senses. The fact that linear acceleration in these cases appears to excite the canals is therefore not a proof that excitation is set up by anything other than a rotatory movement. On the other hand de Kleijn and Magnus (1921) have described what seems to be undoubted stimulation of the labyrinth of mammals by linear acceleration.

Functional grouping of canals. The vertical canals form a functional group by themselves, distinct from the horizontal canals. The grouping, like everything connected with posture or movement, depends upon their relation to the omnipresent and ever acting field of gravitational force.

In any displacement of the body there is either a component in the vertical direction or there is not. If the displacement is indifferent as regards the field of gravity (such displacements are those of rotation on the turn-table, of change of direction in running, trotting, etc.), the body musculature is involved on the whole in a less forcible way than when the animal propels itself against the force of gravity or when its momentum due to gravity has to be arrested (as in the two phases of jumping, or in reactions against a simple fall). The horizontal canals have to do with the former, the vertical canals with the latter. The successive momentary postures mediated by the vertical canals are well seen in photographs of a leaping horse, in which during the ascent the limbs and hoofs are directed backwards, to be thrown forwards during the descent.

This in turn brings us back to the functional correlation whereby each of the four vertical canals is more immediately concerned with the movements of a particular limb. The fundamental influence of a single vertical canal is, by outward thrust of the limb of its own quarter of the body, to give support against momentum acquired through gravity and at the same time to push horizontally towards the center of inertia of the body.

For the proper exercise of this action the head must of course maintain, as far as may be, its normal horizontal orientation.

Posture of head in relation to canal function. That the head should preserve its normal orientation for the proper guidance of movements connected with equilibrium is indicated by innumerable cases. A fowl laid on its back will struggle, but until its neck is raised and its head is held once more in the horizontal plane it performs no righting reaction. All city cabmen know the manoeuvre of sitting on a horse's head, when after a fall his legs have to be disentangled from shafts and harness; once he is allowed to raise his head he is in a position to make struggles to get on his feet. Long-necked animals at rest can bend their head in various orientations; in full career the head is held in characteristically rigid fashion. From this point of view the gravity reaction mediated by the labyrinth must be looked upon as the more fundamental function and as a prerequisite for the proper action of the canals.

The speed of the ampullary reaction. At the same time there is this very striking difference between the two mechanisms. The apparatus for simple maintenance of posture, including pose of the head, in relation to the field of gravity, is slow and deliberate in action, whereas the ampullary mechanism is excessively quick. In our experiments on de-ampullated frogs subjected to fast rotation on the tilt-table, we have over and over again had occasion to note how, in the absence of the ampullary receptors, the proper pose for the inclination is eventually and tardily assumed, but if the animal were to depend during the actual movement on its static mechanism alone, its reactions would invariably be too late. The receptors

of the semicircular canals, being quickly excited, are able to induce a lightning-like response. The consequence is that a normal animal rapidly tilted is at almost any phase of the angular displacement already in a position approximately adjusted for steady maintenance of posture in that position.

Anatomical studies on the cerebellum indicate that incoming afferent impulses have two main pathways to the Purkinje cells; a straight pathway by way of tendril fibres, and an indirect or longer pathway involving the granule cells and possibly cells of the molecular layer in addition. We have no evidence that the ampullary nerves are specially linked with the tendril fibres, but the time-difference in these respective reactions might suggest such a connection. It is more probable however that ampullary impulses are directly relayed by the vestibulo-spinal tract.

Are the canals the only kinetic mechanism of the labyrinth? In a review of the work from his laboratory, Magnus (1924), referring to the effect of displacement of the otoliths of mammals by means of the centrifuge, says, "These experiments do not by any means exclude the theory that the otolithic apparatus also may be excited by movements, indeed this is very probable. But unfortunately we cannot prove it experimentally, because we know of no method of destroying the canals and leaving the otoliths intact." In this connection it may be said that our experiments, in which the canals were neatly destroyed without any damage to the labyrinth, furnish no evidence whatsoever that the otolithic apparatus is stimulated by simple acceleration as such.

In suggesting that the otolithic nervous apparatus is probably affected by movement, Magnus is presumably influenced by physical considerations relating to the construction of the receptor apparatus, for it is impossible to think of an otolith as being unaffected by inertia. It is quite open to suppose however that the epithelial terminals are inexcitable to rapid change and excitable only to steady pressure or tension as the case may be. The fact that there is such a difference in speed of response to ampullary as compared with macular excitation is in itself evidence against the supposition that the otolithic apparatus is stimulated by sudden movement.

THE UTRICULUS. During any operation on the ampulla either of the horizontal or of the anterior vertical canal it was found that if injury occurs in the region of the utriculus, a permanent forced position of the body results. Such injury, entirely accidental, may happen with excessive ease, and the most delicate precautions have to be taken to avoid it. The temperature of the cautery must be just correct and the application must be only for a moment. An operation by section of the nerves is almost impossible. So close does the utricle lie to these ampullae and so slight a traction upon the ampullary nerves suffices to tear or derange it, that at the time of any, even the most carefully conducted, operation on the

ampullae it is impossible to predict the outcome. Only when the frog has recovered from the anesthetic can one say whether the utricle has suffered damage or not. In corresponding operations on the posterior vertical canals the same precautions are not necessary, nor is there the same dubiety as to the outcome. The ease with which the utricle of the frog becomes deranged by the very slightest undue heating or traction upon adjacent nerves, combined with the complete absence of any forced position in successful cases of ampullary ablation, led us to wonder whether plucking out of ampullae with a twist of the forceps as has been practised in dogfish, gives wholly reliable results, though in Maxwell's (1923) hands it appears so to have done.

Unilateral injury. If the utricular injury is on the left, there is an apparent loss of muscular tone on that side; the left side of the body is lowered, the left arm and left leg being flexed, and the body is laterally curved towards the left, the consequence being that the head is inclined to the left and slightly backward. The right arm is strongly extended in the lateral direction, while the right leg is partially extended, laterally and posteriorly. The frog tends to execute forced movements, which are always to the left (circus movements). One must of course keep in mind that the animals we examined with a utricular lesion suffered at the same time from a lesion either of the anterior vertical or of the horizontal canals or of both. A frog with both these canals injured tends, in forward progression, to turn to the left, but the forced circus movements of the laterally inclined frog with a utricular lesion are entirely different from the (more incidental) turning of the normally posed animal that has merely been de-ampullated. It must further be kept in mind that the utricular lesions, the result of accident, were not controlled as regards position or extent, though among the various animals with such injury there were no detectable differences in behavior.

When the frog with a left-sided utricular lesion is turned on its back, it rights itself by rolling over in such a way that its right side is lowermost, this direction of rolling being invariable. In leaping, its hind-limbs are kicked out simultaneously, but in its course through the air it executes a screw or spiral movement, in which its left side first comes lowermost. It usually lands on its back, whereupon, by continuation of the invariable (left-handed screw) direction of rotation about its long axis, it rights itself by rolling over with its right side lowermost, and may jump off again repeating the cycle.

When, on the tilt-table, it is slowly inclined to the left, there is no obvious gravity response. On slow inclination to the right a further extension of the right arm and leg occurs, as if to avoid a return of the head to the horizontal. When subjected to centrifugal force, the animal shows analogous reactions. With its left side towards the center it tilts its body

and is not readily dislodged, but if the right side is towards the center it is more easily dislodged, as it also is on the tilt-table when inclined to the left.

Bilateral injury. If the injury in the region of the utriculus is bilateral, the forced position is symmetrical. The arms are extended laterally, the hands and fingers however still pointing inwards. The legs are partially extended, laterally and posteriorly. The snout is, by flexion of the anterior vertebral joints, markedly depressed, and the wholly unusual attitude of the animal suggests that of a mole attempting to burrow into the ground. Forced movements in the forward direction may occur. In any forward movement the mandibles tend to get caught on the table and the mouth to be pulled open, which fact indicates a lack of tone in the muscles that keep the jaw closed. In jumping, the frog can get clear of the ground, though it does not get the usual upward lift. Our animals too, in which both anterior vertical and horizontal canals were usually functionless owing to associated injury, without any throwing forward of their arms preparatory to landing, slid forward on their belly with arms outstretched.

All gravity responses on the tilt-table are absent. When subjected to centrifugal force with head towards the center, the animal with a bilateral lesion is already prepared to resist the outward throw, but in any other position it is easily thrown against the bell-jar. The response of our animals to quick backward rotation on the tilt-table is unique and characteristic. The hind-limbs, previously somewhat flexed, are sharply extended laterally and posteriorly, so that they become straight. The arms are thrown out laterally, so that the hands come off the table, and the fingers are spread apart. In this position, in which the head still remains flexed, the frog has contact with the table only with its snout and belly. When the backward rotation ceases, the arms and legs are flexed slightly and the hands and feet are again brought in contact with the table. After a bilateral lesion the righting reaction is not wholly lost, though it is gravely interfered with.

The forced positions due to any lesion of the utricle persist for weeks on end with no sign of recovery. No one observing the frogs and knowing the position of the lesion would doubt that the utricular macula is an organ of static equilibrium. It would also seem to play an important part in maintaining the head horizontal.

Analysis of the injury. The permanent forced position of the frog with a unilateral utricular lesion is very like that of an intact animal either after its third shift on a rapidly rotating turn-table or when steeply rotated to one side on the stationary tilt-table. If we may for a moment use subjective language without any of its subjective implications, the horizontal plane to the operated animal feels tilted; towards its sound side the ground has slid from beneath it like dry sand, and the animal is in imminent risk of

slipping; hence too the forced movements—here compare our previous paper. No matter how it turns to get its head upward (circus movement) the stratum continues to slip away from beneath its sound side.

Let us think of a normal frog jumping forward on the side of an incline that dips downwards on its right. During its course through the air its head and body must be held horizontal.. In order to acquire the proper orientation during the free part of its leap, it must, in "taking off," thrust in the upward direction more with its right limbs than with its left; in other words, it has to communicate a momentary adroit and adequate twist of its anatomy to the left, in order to be in proper trim or balance when off the ground. The frog with a unilateral utricular lesion, in taking off from a real horizontal plane, behaves as if it were on an incline; but gravity in this case, instead of acting obliquely to the plane of the substratum, acts normally to it; the consequence is that the initial left-handed twist is overdone and the frog continues while in the air to rotate about its long axis, and lands on its back.

A frog laid on its back, with body-axis horizontal, on an inclined plane of any marked slope, always turns downhill in righting itself. The frog with a unilateral lesion of the utricle, when laid on its back on a horizontal surface, turns with its uninjured side downwards. Thus, in whichever position the animal is placed on the horizontal, whether prone or supine, the ground on the uninjured side is, as it were, felt to be receding from under it; which helps to explain the continued unidirectional rolling movement of many animals with a unilateral labyrinthectomy.

We have ventured to make this attempt at analysis, which may be interpreted mechanically notwithstanding the subjective terminology, because in dealing with the complex problem of posture and movement it would seem advisable to push explanations from existing data as far as they will go. In the process we have chosen not to consider any possible influence of coincident damage to the two anterior ampullae. While such damage may contribute in a minor, it certainly does not contribute in any major degree, to produce the observed results. While it is tempting to pursue the subject still further, especially in relation to the direction of the receptor stimulation and the central connections of the receptors themselves, we prefer to leave it with the statement that any attempted analysis of the permanent posture after a bilateral is necessarily far more difficult than after a unilateral lesion. In the end we were at no time sure of the extent of the utricular lesions.

THE SACCULAR MACULA AND OTOLITH. Before the ablation experiments were undertaken the illustrations of Retzius (1881) were used as a guide to the anatomy of the frog's internal ear. The dissections were first carried out on the bull-frog, several exposures of the saccular chamber and otolith being made. One specimen, in which an opening was made into the

saccular chamber from below and behind, was, after formalin treatment, left exposed to the air. On examination after the chamber had dried out, the otolith was seen to be surrounded by the perilymphatic space, as described by Retzius, but the macular membrane on which the otolith rests was seen to be attached along its free margin to the wall of the chamber by means of a number of fine threadlike stays. This discovery in turn led to an investigation of the anatomy of the macula.

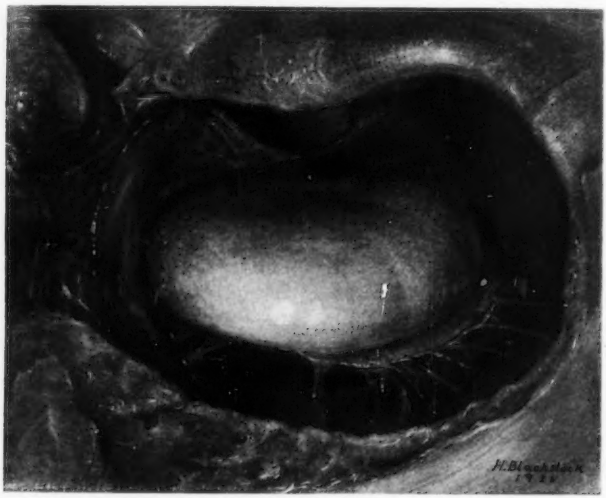

Fig. 2. The right saccular chamber of Rana catesbiana opened from below and behind. Drawing. ×24.

The specimen is so adjusted that the membrane is nearly horizontal. The saccular otolith is seen to cover the saccular membrane, except for a narrow projecting shelf towards the front and right. The border of the membrane is attached by (horizontal) stay-threads to the wall of the saccular chamber (towards the front some of these threads have been broken in preparation). The membrane is supported below by thicker struts or pillars, those towards the front being vertical, the lateral ones oblique (some of the pillars in front were also broken in preparation). In the upper left-hand corner is seen the ampulla of the posterior semicircular canal. Above the otolith, in the middle, is seen the opening of the perilymphatic duct.

Figure 2 is an illustration of the appearance presented in a labyrinth so arranged that the macular membrane is approximately horizontal. The smooth and somewhat hemispherical otolith, which consists of a thick creamy substance inclosed in a thin capsule, is seen to rest by its flat surface upon the macular membrane, to which the capsule is attached beneath. The edge of the membrane projects like a shelf from beneath the otolith. From its free border numerous delicate threads (about 14 in an intact speci-

men) pass across the perilymphatic space to gain attachment to the wall of the saccular chamber. In addition to these threads, thicker pillar-like structures (likewise shown in the illustration), arranged vertically below the membrane, give it rigidity and support. Further dissection shows that these pillars are arranged somewhat regularly around the distribution of the nerve to the macula.

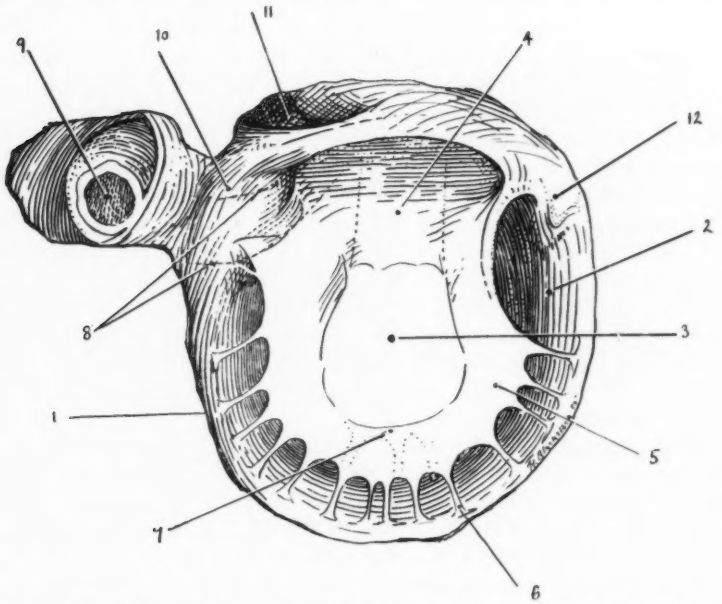

Fig. 3. The left saccular macula, after removal of the otolith with its capsule, as it appears in situ when viewed from above and laterally. × 24.

1, The lining membrane of the saccular chamber. *2*, Perilymphatic space. *3*, Macula lying over the insertion of the macular nerve. *4*, Outline of the saccular nerve coming from the upper medial wall. *5*, Shelf of the macular membrane which projects beyond the otolith, and to which most of the stay-threads are attached. *6*, Stay-thread. *7*, Outline of a few of the supporting pillars, which are attached around the edge of insertion of the saccular nerve. *8*, Outline of the ramus acusticus anterior. *9*, Interior of the ampulla of the left horizontal semicircular canal. *10*, Wall of the utriculus. *11*, Opening of the perilymphatic duct. *12*, Wall of the lagena.

It thus appears that the encapsuled otolith material rests, as it were, upon a blanket stretched out by many hands and further supported by struts from below. Figure 3 shows the saccular membrane after the otolith has been removed. Here it may be observed that the membrane at one end (upper and medial in the head of the animal) passes under the archway formed by that portion of the saccular wall to which the otolith

capsule is attached. Having passed under the archway, it becomes continuous with the wall of the utriculus. The membrane is thus connected by thread-like stays only along part of the border. The pillars that support the membrane (which latter we may for a moment compare with the human tongue) are, near the tip, perpendicular to it; laterally they become increasingly oblique as one passes back towards the base. There are in all some 15 or 20 of these pillars. The membrane which thus supports the otolith projects shelf-like from beneath it only towards its tip. Laterally the otolith capsule fuses directly with the edge of the membrane, figure 2.

In his description of the saccular otolith of the frog Retzius mentions that on its under surface there are "gefässführende Brücken am Periost befestigt," and in his illustrations a few irregular lines are shown on the inferior aspect of the otolith. These "vessel-carrying bridges" are probably the pillars here described, but Retzius makes no mention either of their regular arrangement or of the thread-like stays by which the membrane is attached. When one considers the architectural arrangement and the number of these pillars, it is unlikely that the function attributed to them by Retzius is correct. When the saccular chamber is opened from below, so that the under surface of the macula is exposed, a relatively large vessel is seen to run in along the saccular nerve; it divides into two main branches, which spread out and subdivide over the under surface of the macular membrane.

Shambaugh (1913) and Nabeya (1923), describing the blood supply of the mammalian internal ear, state that the arterial branch to the saccule comes from the *arteria vestibuli posterior* at the base of the cochlea. Shambaugh refers to several small arteries that supply the epithelial surface of the macula. In the case of the frog, in which the macula is not in contact with the wall of the saccular chamber, the vascular arrangements appear to differ from those in the mammal. de Burlet and de Haas (1923) describe the saccular macula in the mammal without referring to any pillars or stay-threads, nor does Maxwell (1923), discussing the anatomy of the saccular macula in the dogfish, make any mention of such structures. The probability is that the arrangements differ in different animals.

In the frog the saccular nerve, after leaving the *ramus acusticus anterior* —figure 3, *8*—enters the saccular chamber at the upper and inner wall just below the base of the macular membrane—*4* of the figure—and is distributed over the central third of the macular membrane—*3* of the figure.

Figure 4 is an attempt to represent diagrammatically the places of direction of the saccular maculae (or saccular membranes) relatively to the body. The diagram represents a coronal section through the frog's head at the level of the saccular chambers, the section being viewed from behind. The plane of the left saccular macula is represented by the square on the left side, and that of the right saccular macula by the pentagon on

the right. The upper surface of each macula looks backwards, outwards and upwards. With the vertical sagittal plane the plane of the saccular membrane makes a vertical angle of some 22 degrees, and a horizontal angle of some 27 degrees. The actual size of the saccular macula in a large bull-frog is about 2 mm. in diameter. The saccular membrane, supported by its pillars, is about $\frac{3}{4}$ mm. distant from the inner wall of the chamber. The stay-threads are about $\frac{3}{4}$ mm. long.

Ablation of maculae. Functional elimination of one or of both saccular maculae is an easy operation. A rather doubtful and uncertain method of abolishing function is to slit the capsule of the otolith and to wash out the creamy otolithic material; a reliable method is to sever the saccular nerves. After section of the nerve to one or to both maculae the animal sits, crawls, jumps and swims in perfectly normal fashion; there are no forced positions. All the responses on the tilt-table are normal; so too are the reactions when the frog is subjected to centrifugal force on the turn-table. The responses to quick rotation (angular acceleration) about all axes are normal. As it is impossible to demonstrate any disturbance of posture or of movement after either a unilateral or a bilateral section of the saccular nerve, it is plain that in the frog the saccular macula has no connection with equilibrial function. These findings are in accord with those of Laudenbach (1899) on the frog and with those of Parker (1909) and of Maxwell (1923) on the dogfish.

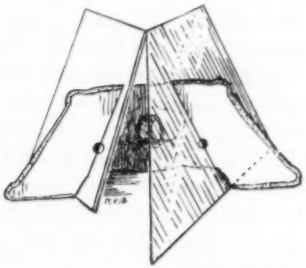

Fig. 4. Diagrammatic coronal section of frog's head at the level of the saccular chambers, seen from behind. The quadrilateral and pentagonal figures represent the plane of the saccular membrane on the left and on the right side respectively. Each membrane faces backwards, outwards and slightly upwards.

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SUMMARY

1. Ablations of individual ampullae of the semicircular canals have been effected. By systematic examination of the reactions of the de-ampullated animal to gravity and to centrifugal force, to linear acceleration and to angular acceleration about various axes, the resulting disturbances of function have been determined.

2. The semicircular canals are not involved in static reactions in gravity or to centrifugal force, nor does their absence entail forced movements or forced positions. They respond only to acceleration, and the reactions due to their stimulation are compensatory movements, whose duration approximately equals the duration of the exciting acceleration.

3. The horizontal canals seem to respond only to angular acceleration. The canal of one particular side is excited only when the direction of acceleration is such that the narrow part of the tube is made to move in advance, the ampulla bringing up the rear; it does not respond to angular acceleration in a direction which makes the ampullary end move in advance.

4. Any given vertical canal would seem to respond to angular acceleration (in its own plane) either in a positive or in a negative direction; yet that direction of acceleration which causes the ampullary end to move in advance is by far the most effective stimulus.

5. Effective stimulation of one horizontal canal brings the musculature on both sides of the body into play. Each vertical canal on the other hand is mainly concerned with the musculature of its own side.

6. The geometrical disposition of the four vertical canals is related to the four "quarters" of the body each with its own limb; for example, the left anterior vertical canal is especially concerned with movements of the left fore-limb, and the left posterior canal with movements of the left hind-limb.

7. The fundamental influence of a single vertical canal is, by outward thrust of the limb of its own quarter of the body, to give support against momentum acquired through gravity, and at the same time to push horizontally toward the center of inertia of the body. Thus the four vertical canals form a functional group distinct from the horizontal, the action of which bears no particular relation to momentum acquired through gravity.

8. Frogs with lesions of the vertical canals, when subjected to linear acceleration by movement of the surface on which they rest, show abnormal reactions identical with those exhibited when the same animals are so tilted that they tend to fall in a direction opposed to that of the particular linear acceleration which produces the abnormal response. The probable reason is that "their feet are pulled from under them" and the labyrinth is thereby subjected to an angular acceleration.

9. Proper function of the canals presupposes a horizontal orientation of the head by means of the more fundamental otolithic apparatus.

10. The reaction to ampullary stimulation is characterized by great speed as compared with the static reaction to otolithic stimulation.

11. The canals are the only mechanism of the labyrinth stimulated by acceleration.

12. In the frog the utricles are the great labyrinthine apparatus for static equilibrium. A frog with a unilateral utricular lesion assumes a

permanent forced position and may show forced movements. In jumping it makes spiral or screw movements. Its gravity responses are characteristically interfered with. A frog with a bilateral utricular lesion gives no labyrinthine gravity responses whatsoever.

13. The forced position and movements, the spiral leaping of the frog and the rolling of some animals about their long axis, as the result of a unilateral utricular lesion, are analysed and shown to depend on the principle that, whether the animal is prone or supine on the horizontal plane, the ground, as it were, recedes from beneath it on the sound side.

14. The saccular otolith of the frog rests upon a macula-bearing membrane which, projecting like a shelf into the saccular chamber, is suspended by stay-threads and supported by pillar-like structures.

15. After section of the nerve of one or of both saccular maculae of the frog there is no disturbance of posture or interference with reactions to acceleration. Whatever its function, the saccular macula of the frog is not concerned in equilibrium.

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A PLETHYSMOGRAPHIC STUDY OF THE CHANGES IN THE VOLUME OF THE SPLEEN IN THE INTACT ANIMAL

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As all previous studies on changes in the volume of the spleen have been made either in the anesthetized or dead animal and are consequently complicated, it seemed to us that additional data might be obtained by studying these changes in the intact animal.

A review of the known anatomic facts concerning the spleen reveals two which may be of considerable importance with regard to the functional significance of the organ. As suggested by Gray (1854) and emphasized by Mann (1921), the spleen is the largest mass of lymphoid tissue in the body, and the splenic vein drains into the portal circulation in all species of animals in which this point has been determined.

The spleen as the largest mass of lymphoid tissue in the body should have an important functional relationship with the general hematopoietic and reticulo-endothelial systems, and the relation of the spleen to the portal circulation suggests that its function might be intimately associated with that of the liver or the gastro-intestinal tract, or both. One of the first functions ascribed to the spleen emphasized the possibility of this relationship. In this regard it should be noted that the spleen can act as a circulatory shunt, whereby the portal capillaries of the liver may receive blood that does not come from the digestive tract, which is laden with the products of digestion.

One of the most marked characteristics of the spleen is variation in size. Many of the results of the investigations on the spleen which depend on a measurement of its size are therefore of little value. With the exception of the known facts with regard to the relation of the spleen to the hematopoietic and reticulo-endothelial systems, its variation in size during digestion and the possible relation of this change to the digestive processes offer the most important suggestion as to its possible functions. We therefore undertook a series of experiments in an attempt to determine whether there was any definite demonstrable relationship between the activity of the spleen, as manifested by changes in volume, and the activity of the gastro-intestinal tract.

REVIEW OF PREVIOUS INVESTIGATIONS. Dobson (1830) showed that the spleens of animals given heavy meals of beef and mutton varied considerably in size at different periods after eating. He found little change in volume until about the fourth hour, after which it seemed to increase until near the fifth hour, and then gradually to decrease for twelve hours. He believed that the spleen acted in the capacity of a storage chamber for blood, and by its elasticity protected the vascular system from shocks brought about by sudden changes in pressure or increases in the volume of the blood during digestion. He cited as an example of the former the spleen of a malarial patient which receives a surplus of blood during the rigor, as a result of which there is an increase in the volume of the spleen and a stretching of the capillaries beyond their ordinary capacity. Successive influxes of blood before the capillaries can resume their natural condition eventually destroy their contractility. Enlargement of the organ is the natural consequence, disease is set up and a chronic state of thickening and hypertrophy established. Dobson found that the injection of blood into the jugular vein caused an increase in the volume of the spleen and that bleeding caused a decrease in volume. Contractions of the spleen of the dog were first shown by Wagner (1849), and three years later Henle (1852) showed the presence of contractility in the spleen of man. Schiff (1867) observed contractility in the spleens of rabbits and cats on stimulation of the splanchnic nerve and semilunar ganglion. Sabinsky (1867) found that after death of an animal from asphyxia this organ was constantly contracted, and concluded that this effect was a nervous one and not due to the direct action of the asphyxial blood on the organ. Oehl (1869) obtained contraction of the spleen by stimulating the peripheral end of either vagus in the neck of dogs, rabbits and cats. Bulgak (1877) and Tarchanoff (1874) found that stimulation of the medulla caused a contraction of the spleen. Roy (1881) was the first observer to use a plethysmograph to record the changes in the volume of the spleen. He used anesthetized animals for his experiments. He showed that the spleen of the dog was constantly undergoing rhythmic contractions. These contractions were exceedingly regular in rate, but subject to many variations in extent, especially at the appearance of the Traube-Hering curves of the blood pressure, the conflict between the smaller Traube-Hering curves and the larger splenic waves producing a result unlike either and called by him "interference waves." Concerning the volume of the organ he asserts that this is subject to endless variations and gives one example in which a variation of 36 per cent was noted after death. He confirmed the results of Bulgak on stimulation of the medulla and added that stimulation either of the central end of a cut sensory nerve, or of the peripheral ends of the vagi or splanchnics caused a contraction of the spleen; but that after section of the vagi or splanchnics, stimulation of a

sensory nerve still caused a diminution in the volume of this organ, and concluded that vasomotor influences might travel by some other route than by the nerves named. Schäfer and Moore (1896) found that the spleen was quite responsive to changes in blood pressure and believed that the rhythmical contractility of the organ was inherent and might be carried out entirely independently of the central nervous system or of the large sympathetic ganglia. After painstaking dissections they found that the muscular tissue of the spleen was innervated through an extraordinarily large number of nerve roots, the largest outflow coming from the fifth to the ninth postcervical inclusive. The outflow is not symmetric, being greater on the left than on the right, and, further, there is probably a cell connection in the main ganglionic chain of the sympathetic and perhaps another in the semilunar ganglion. They believed that the spleen received both motor and inhibitory fibers through the splanchnics but that the pneumogastric contained no fibers which directly influenced the contractions of the organ; further that the spleen was very responsive to the products of asphyxia, which produced not only a general contraction of the organ but also an increase in its rhythmic movements. They obtained marked contraction of the spleen with epinephrin and concluded also that certain drugs and extracts had a specific action in increasing the extent of the movements, for example, curare and brain extract. A review of the large amount of work on the relation of the spleen to digestion was made by Inlow (1921). He investigated the effect of splenectomy on the secretion of gastric juice, pancreatic juice and bile. His results conclusively prove that the spleen does not affect the digestive capacities of these secretions.

This review of previous work readily shows some very suggestive data pointing to a relation between increased activity of the spleen with increased activity of the gastro-intestinal tract. However, the methods of recording the changes in the volume of the spleen, such as measuring the organ after death, or changes in volume under anesthesia, offer a reasonable doubt of the physiologic significance of this relationship.

Method of experimentation. In our work on the changes in the volume of the spleen in relation to digestion and other processes, it was necessary to develop a technic which would permit of repeated observations on animals and in which the results would not be complicated by the effects of an anesthetic. A plethysmograph was made of nonflexible collodion and a technic was developed making such a study possible. A detailed description of the preparation of the plethysmograph and mode of application as well as the method of recording the results was given in another paper (Hargis, 1925). The animals used have been dogs in all instances. All operative procedures were carried out under complete ether anesthesia and with a rigid sterile technic. In applying a plethysmograph which is to remain in the abdominal cavity for long periods of time

it is necessary to exercise the utmost care in every detail of the technic if success is to be obtained.

During observations the animal is placed on a concave padded holder, on its side, with a collar around the neck and the feet loosely restrained. Appropriate connections are arranged between the tube from the plethysmograph and the recording apparatus. Observations are made only on animals trained for the purpose and maintained in a very comfortable position. At the expiration of the first post-operative week they are placed under preliminary observation for increasing periods of time so that they will become accustomed to the surroundings and acquire the ability to lie quietly on the table. Dogs are easily trained for this work and quite readily learn the routine expected of them; the trained animal will remain perfectly contented for a long time, frequently sleeping while the observations are being made. The method of recording was that developed and described by Potter (1924).

The changes in the volume of the spleen were studied in relation to diet, drugs, reflex response to different stimuli, and the effect of rebreathing. In selecting animals for dietary experiments it was found that those termed "good feeders" gave better results than those endowed with more delicate and discriminating appetites. The animals were studied under a fixed technic; they were first thoroughly studied while receiving the mixed diet of the laboratory, then for periods of one week while they were fed on diets containing standard quantities graded to the body weight. It is important to be thorough in the preliminary observations in order that the idiosyncrasies of the different animals may be understood. Before observation the animal is fasted overnight and in the morning a tracing of the movements of the spleen in the fasting condition is taken. While the animal is still on the table and connected to the recording apparatus it is fed the required amount of the special food, and as soon as it becomes quiet a continuous tracing is taken throughout the first hour and then at hourly intervals, eight hours, unless a longer period is indicated.

All of the drugs studied were administered intravenously except amyl nitrite, which was introduced into the rebreathing apparatus and will be described in connection with that. Drugs have been administered both by the jugular and saphenous veins, but in either case the injections were made with as little difficulty and manipulation as possible.

Some difficulty was encountered in increasing the carbon dioxid content in the blood in the intact animal, as it was desired to produce vasomotor as well as respiratory stimulation and essentially by some method which would not cause the animal discomfort or make it struggle. This was accomplished by using the mask of the type employed as a routine in metabolism work on the dog in our laboratory as described by Kitchen (1924). This mask was connected with a closed system of small

capacity which contained a larger rubber balloon to care for the individual respiratory excursions. The neck of the dog was shaved closely and the mask fitted in place. Wide rubber dam was wrapped about the posterior portion of the mask and neck until an air-tight joint was obtained. There are two openings in the upper end of the mask to which connections may be made; one of these was closed and the other connected to a rubber tube of large caliber. A large water bottle, of about 20 liters capacity, was fitted with a double perforated stopper into which two pieces of glass tubing were inserted. A rubber balloon was attached to one glass tube and the rubber tube from the mask to the other. The animal was placed on the table, the mask applied as described and a tracing made with both apertures of the mask open. After sufficient control has been obtained the connections were made and rebreathing allowed to progress until the desired effect was produced. This method was found to be very satisfactory.

The effect of amyl nitrite was studied by closing one aperture and breaking a standard pearl into the mask during a period in which a continuous tracing was being made.

In order to aid in the analysis of the curves and to have control observations to obviate the possible mechanical errors complicating our observations, two series of experiments were performed in which the spleen or a portion of the organ was denervated. In the first series the plethysmograph was applied in the usual fashion and several observations were made. Then under ether anesthesia and with sterile technic the nerves to the spleen were severed and after a suitable period the observations were repeated. In the second series the spleen was divided in two as nearly equal portions as possible and the nerves to one-half destroyed and left intact in the other. Each half was placed in a separate plethysmograph. Observations were then made according to the usual routine and the changes in volume taking place in each half of the spleen recorded synchronously.

The preparation of the divided spleen, though it requires rather exact technic, is not a very difficult procedure. Plethysmographs, of the same diameter but about half the usual length, with ledge and aperture for vessels, are prepared and applied by the general method previously described for the regular instruments.

The normal arrangement of the splenic vessels is such that the organ may be divided between branches and the blood supply remain practically as good as before interference. The splenic artery normally divides and sends a branch to be distributed to each half of the organ, and clamps can be placed across the middle portion of the organ between the points of entrance of the two main branches. There should be practically no loss

of blood, and the subsequent healing is generally satisfactory. Two large crushing clamps are placed across the organ, dividing it as nearly as possible into two equal portions and the organ cut between the clamps. Two rows of continuous catgut sutures, one placed before and the other after removal of the clamps, will control the bleeding. Care must be taken that the sutures are placed only in the crushed tissue; otherwise troublesome hemorrhage may be encountered from needle punctures in the normal tissue. Good results cannot be obtained if plethysmographs are applied to an oozing organ.

One portion is selected for nerve section; the results are the same regardless of which segment is used. The vessels are exposed as far as the bifurcation of the splenic artery. The nerves are to be found in the tissue surrounding the vessels and all of this is carefully dissected away until the vessel walls are clean. Segments are then cut out of the nerve branches. Very small nerve filaments often adhere quite closely to the vessels and unless the dissection is carefully and completely done some of these may be left intact. When all of the nerves have been sectioned the portions of the organ may be placed in their respective plethysmographs. The omentum is not large enough to cover both portions of spleen and the two plethysmographs, so it is necessary to introduce the organ without omental covering in order that there will be sufficient omentum to encapsulate the instruments thoroughly. However, there will be thickening of the capsule and such animals cannot be used for observations for as long periods as normally, though many of them have been studied for periods of from four to six weeks. The rubber tubes are carried through stab wounds in the abdominal wall. Sometimes it is better to bring one tube out through the incision, the criterion being that there shall be no torsion of the vessels.

Results. *Spontaneous changes in the volume of the spleen.* The changes in volume were more active and greater than we anticipated. The spleen of the normal dog almost constantly undergoes spontaneous changes in volume which are remarkably constant, at a given time, in rate and amplitude. These changes are practically always present to some degree and the resulting curves are usually very regular (fig. 1). The most marked variation occurs in the amplitude, although the rate may also vary. Quite frequently the effect of the heart's beat and respiration will also be recorded with the variation of splenic volume. The curve produced by the variation of splenic volume is rather characteristic. The increase in volume takes longer than the decrease. A measurement of the time of 100 curves taken under different conditions, showed that the average time for a complete cycle was forty-seven seconds, and of this twenty-seven seconds represented the time the spleen was increasing in size and twenty seconds the time the organ was decreasing in size. Although the

curves due to the spontaneous changes in the volume of the spleen are quite similar, individual variations occur. This necessitated a careful control study of such movements in all animals.

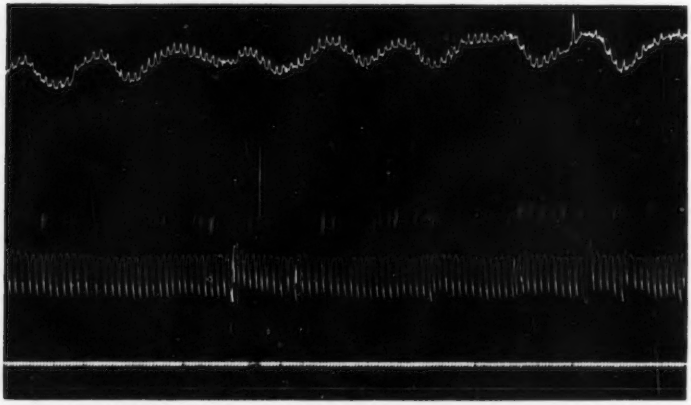

Fig. 1. The normal or spontaneous variations in the volume of the spleen in the fasting intact animal. From above downward are shown tracings of splenic volume, respiration and time in minutes and seconds.

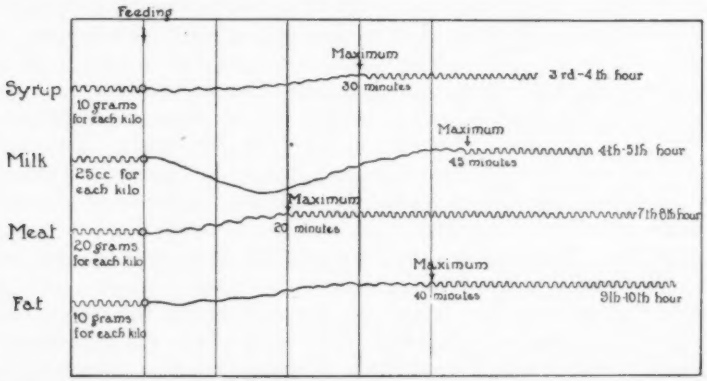

Fig. 2. A diagrammatic representation of the changes produced in the splenic volume by selected diets. The greatest changes were produced by a diet of meat and the least by carbohydrates.

The effect of diet on the changes in the volume of the spleen. The effects of fasting and of various diets on the changes in the volume of the spleen were studied. Carbohydrate, fat, protein, and milk diets were used (fig. 2).

There is considerable variation in the spontaneous changes in the volume of the spleen in different animals. One may present undulations which are extremely regular or rhythmical over long periods of time while another will present changes in extent as well as rhythm which vary within much greater limits. Similar variations occur in different animals in the fasting stage and after feeding. Some animals in the fasting condition will present minor changes which are quite regular and of a constant extent, while in others there may be a period in which changes do not appear for a time, only to be followed by one or two well-marked cycles. The changes in volume in the fasting stage, however, are generally of minor extent, and present not only an arrythmia but also a disposition to disappear almost entirely at times. The rate of the individual cycles, however, is much the same as that during the period after feeding.

Corn syrup was given for the carbohydrate food. Animals were fasted over night and placed under observation the following morning when tracings were taken of the changes in the volume of the spleen during fasting. While still on the table and connected to the manometer, the animal was given 10 grams of corn syrup for each kilogram of body weight and a continuous tracing taken during the first hour and at hourly intervals for eight hours. At first the changes from the fasting condition were not marked but after a few minutes the curves began to increase in amplitude and seemed to reach their maximum about thirty minutes after feeding. These maximal changes were not so marked as those obtained with the other diets and lasted until about the fifth hour after which they began to decrease progressively until the fasting stage.

Lard was given as the fat food. After the same preliminary management the animal was placed on the table and connected to the manometer. Ten grams of melted hog lard for each kilogram of body weight was fed and a continuous tracing taken during the first hour and at hourly intervals for ten hours. There was a gradual increase in splenic volume, and rather marked changes appeared early which were quite rhythmical. These continued to increase in extent until the maximum was reached about forty minutes after feeding. The maximal changes were observed until the ninth or tenth hour, after which they began to decrease.

Meat and meat broth were given for the protein food. Of all the diets studied the greatest activity in the changes of the spleen was observed with meat. Twenty grams of lean beef for each kilogram of body weight was given and a continuous tracing taken during the first hour and then at hourly intervals for eight hours. There was a gradual increase in the volume of the spleen, and the changes were greater from the beginning than with any of the other diets studied. The maximum was attained in about twenty minutes and continued until about the seventh or eighth

hour, after which the variations began to decrease progressively until those of the fasting stage appeared.

If the animal is given meat broth under routine conditions and allowed to become quiet the changes in volume will appear quickly. They will not be as great in extent as with whole meat and there will not be the immediate rise, but the activity is greater than with milk or water. The maximum is reached later than with whole meat and the decrease in extent appears earlier.

As an example of an easily administered mixed food the effect of milk was studied. After the usual preliminary procedures the animal was given 25 cc. of whole milk for each kilogram of body weight and allowed a few minutes to become quiet, after which a continuous tracing was taken for one hour and then at hourly intervals for eight hours. During the first few minutes there was a slight decrease in the volume of the spleen and the variations were very slight, at times almost absent. This period was followed by a very gradual increase in volume, and minor fluctuations in the curve were seen. During this period the cycles were poorly marked but rhythmical, and continued with gradually increasing intensity until the maximum appeared about forty-five minutes after feeding. The curve showed the greatest amplitude until the fourth or fifth hour after which it decreased until the level of the fasting stage was reached. No marked differences were noted in the results whether the animal drank the milk or whether it was given by tube.

Water as a control, given to the same animals in like amounts and under similar conditions, caused a gradual increase in splenic volume without the initial decrease in volume, and cyclic changes were practically the same as those of the fasting stage.

Psychic effect of tempting a hungry animal with food. The psychic effect of tempting a hungry animal with food is of interest as it illustrates another characteristic modification in the cycle. While the animals after having fasted for eighteen hours were having the preliminary tracings taken meat was very quietly placed by an assistant where the animals could inhale the odor. The undulations of the fasting stage were replaced by waves of shorter duration and lesser amplitude, which continued for a variable period of from a few to several minutes.

The effect of drugs on the volume of the spleen. We have not attempted a comprehensive study of the effect of drugs, but of those employed some were selected because their action might be more likely to affect the volume of the spleen on account of its great vascularity, and others because their actions were so marked and definite that they could be used as controls for our method of study. By adopting a routine for the intravenous injections and training the animals for the procedure, drugs may be introduced with very little difficulty and the animals will remain quiet. The jugular

or saphenous vein can be used; the former is preferable because of its size. A large area of skin over the vein is shaved quite clean. A preliminary tracing is taken and then the needle is introduced into the vein by an assistant. Generally with the introduction of the needle there is a sudden decrease in the volume of the spleen, but it returns quickly to its former state. The needle is held in place until the changes in volume appear normal or similar to those obtained before the manipulation; then the injection is made and the needle quickly withdrawn. The customary average dosage of the different drugs for each kilogram of body weight was employed.

The spleen responds very markedly to action of epinephrin. It was found that 0.01 cc. of 1:1000 solution for each kilogram of body weight would produce a very great and prolonged decrease in splenic volume.

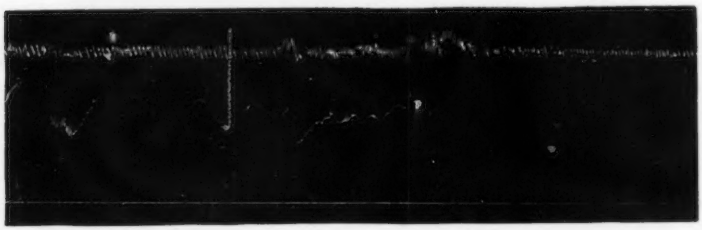

Fig. 3. Variations in the volume of the spleen with simultaneous tracing of the blood pressure. The cannula of the mercury manometer was introduced into the carotid artery under local anesthesia. A rather sudden decrease in volume occurred when the needle was introduced into the jugular vein and a slight extravasation of adrenalin solution prolonged it. The tracing shows the effect of the intravenous injection of 0.01 cc. of 1:1000 solution of adrenalin for each kilogram of body weight on splenic volume and general blood pressure in the intact animal.

The effect seemed out of proportion to that produced on the general blood pressure or the volume of the kidney (fig. 3).

The intravenous injection of 0.1 cc. of pituitrin for each kilogram of body weight produces quite a marked decrease in the volume of the spleen. The response of this drug is similar to that obtained with epinephrin. The change in volume is not so marked, nor does it occur so quickly, and the effects last longer.

The method of administering amyl nitrite has been described. With small amounts a decrease in the volume of the spleen was produced which was quite gradual while the fluctuations in the volume of the organ continued. These changes were of somewhat shorter duration but were well marked in extent. Larger doses of the drug produced a rather sudden and marked decrease in the volume of the spleen quite similar to that obtained with small doses of epinephrin (fig. 4).

Effect of rebreathing. The method used for producing the effects of

rebreathing has already been described in some detail. It consists essentially of having the animal breathe into a closed system of small capacity until the products of respiration have accumulated sufficiently to produce the required effects but without discomfort to the animal or causing it to struggle. With an animal trained for the work and arranged as described,

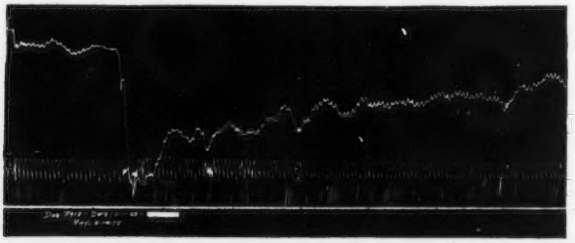

Fig. 4. The effect of breathing small quantities of amyl nitrite on the volume of the spleen in the intact animal. The drug was introduced into the rebreathing apparatus and the amount of air controlled by adjusting the valves of the apparatus. There was a gradual decrease in volume but the rhythmic variations continued throughout.

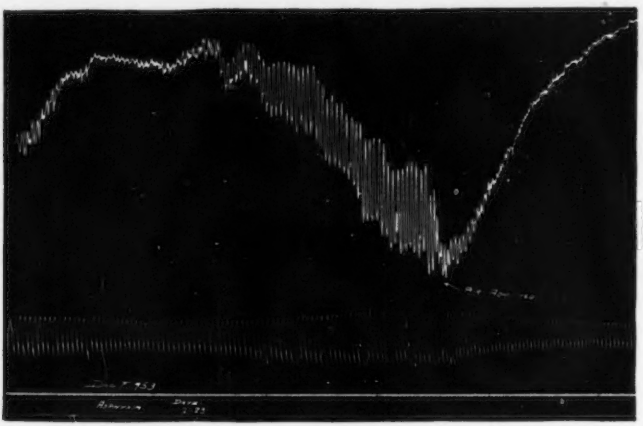

Fig. 5. The effect of rebreathing on the volume of the intact spleen. There is a gradual decrease in volume as rebreathing progresses and a return to previous volume when air is admitted.

it was found that the spleen was profoundly influenced by rebreathing. The volume of the organ decreased gradually as the respiratory products accumulated, and when air was admitted the organ gradually returned to its previous volume (fig. 5). That the decrease was proportionate to the accumulation of the respiratory products was shown by the fact that if

the system contained carbon dioxid when the experiment began, the result was attained much more rapidly than if the system contained normal atmosphere. The greater the quantity of carbon dioxid, the greater was the decrease in the volume of the spleen, up to a certain limit.

The splenic reflex. Although this series of experiments was undertaken for the purpose of determining the changes in the volume of the spleen in relation to the activity of the digestive tract, the observations of another phenomenon were productive of greater interest. It was noticed that, during observations on the first animal of the series, when anyone entered the room, there would be an instantaneous momentary decrease in the volume of the spleen, usually followed by an increase in the rhythmic changes in volume. This observation led to an intensive study of what can be termed the splenic reflex (fig. 6). It was found that a very wide variety of stimuli would produce such a decrease in splenic volume.

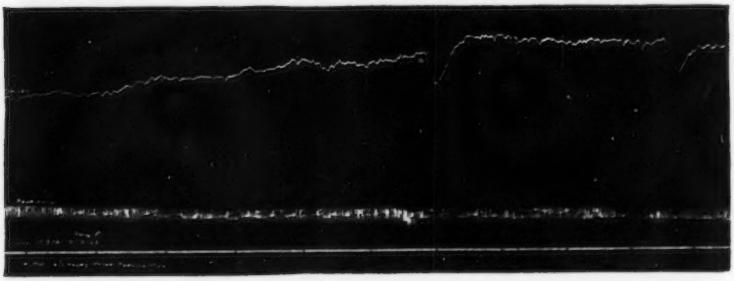

Fig. 6. The splenic reflex. There may or may not be respiratory variations accompanying the reflex change produced by external stimuli. Two reflex changes are shown, the first of which has an accompanying respiratory change while the second, which is just as great in extent, shows none.

Among these can be mentioned any loud noise, as the clapping of the hands, the sudden starting of a motor, the banging of a door, the ringing of the telephone; procedures involving some discomfort to the animal, as slight pinching of the tail, applying drops of cold water to the abdomen, blowing smoke into the nostril, and many other similar procedures. Fright was probably the most prominent emotion associated with these various procedures. This splenic reflex has been observed in every animal studied, although there has been a wide range of variability in the reflex in the different animals. The reflex was so easily induced in many of the animals that the effect of diet and drugs was studied with the greatest difficulty. In many instances it was found necessary to carry on the observations in a closed room from which all extraneous noises had been excluded. Even this was not sufficient with some of the animals, and in these cases investigations had to be carried on at night when the

laboratory was very quiet. On the other hand a few of the animals gave very little response in regard to changes in splenic volume following various external stimuli.

Many interesting observations were made with regard to the efficacy of the different stimuli and the effect of repeated stimuli on the splenic reflex. It was noted that in some of the animals a certain stimulus might cause a marked reflex each time it was employed. In other animals, however, a definite stimulus caused a marked reaction the first time it was

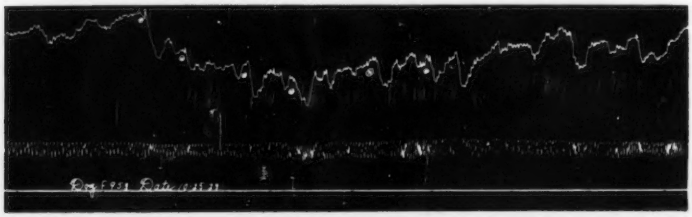

Fig. 7. Reflex variations produced by a series of external stimuli, as shouting, whistling, and clapping of the hands.

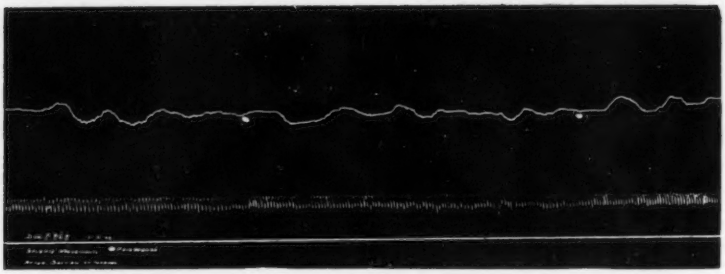

Fig. 8. Spontaneous variations in the volume of the spleen after denervation. The rhythmic changes continue but are less marked than with intact nerves. The reflex is lost after section of the nerves. The signs (*) indicate points of stimulation but the sudden reflex decrease in volume does not occur as it did in the same animal before section of the nerve.

used but thereafter produced no effect during that period of observation; or a certain stimulus behind the animal would produce a characteristic result, not evidenced if the assistant stood where the animal could see him and anticipate his actions. Surprise was often essential. The alert, intelligent type of animal became accustomed to the different procedures and after several repetitions the reactions were more and more difficult to obtain. It was not that the reflex could not be elicited but that new kinds of stimuli were needed to produce the effect in the well-trained animal. On the other hand a few animals always responded with a change in

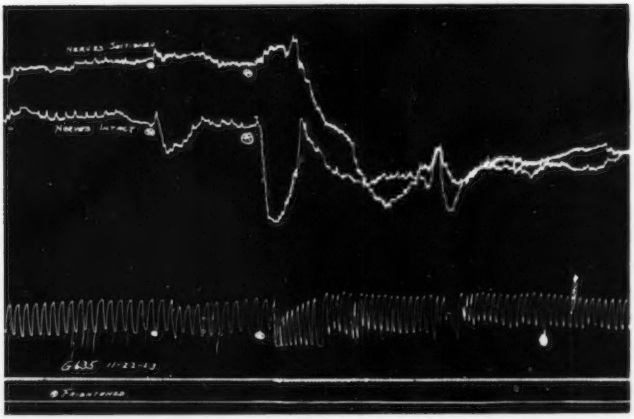

Fig. 9. Reaction of the divided spleen to fright. The reactions obtained with the intact and denervated whole spleens are obtained simultaneously in the divided spleen, which eliminates the possibility that mechanical factors are important in the production of the changes. The intact portion decreases in volume and the denervated portion increases slightly, and after a few moments both decrease equally. This secondary change may be accompanied by a change in the general blood pressure and may bear some relationship to the emergency function of the adrenals.

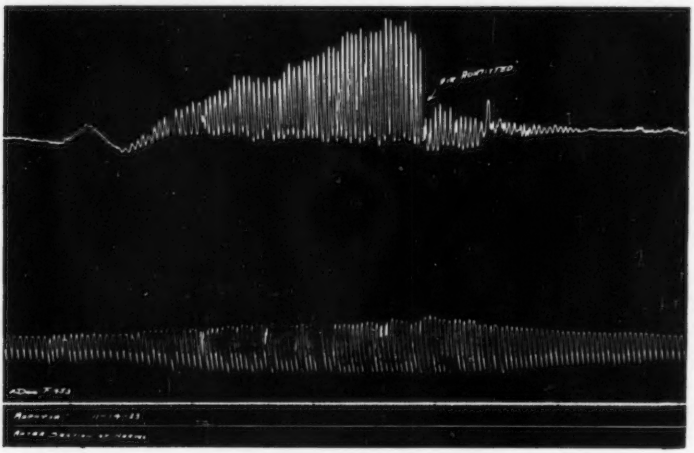

Fig. 10. The effect of rebreathing on the volume of the denervated spleen. After nerve section rebreathing produces an increase in volume instead of a decrease as in the intact organ. Compare with figure 8.

splenic volume, even though they were under observation for a long time and were fully cognizant of the different procedures.

The decrease in splenic volume following these various procedures varies to a considerable extent with the strength and character of the stimulus, and is generally greater when the animal is unable to see the agent which produces the stimulus. A very slight sound will not cause as great a decrease in volume as a loud crash, although the reaction may be similar. By using an animal which is not well trained, it is possible, by eliciting the reflex repeatedly, to decrease the volume of the spleen beyond the limits of our method of recording (fig. 7).

Changes in the volume of the denervated spleen. In order to control our observations with regard to mechanical errors, especially those caused by the splenic reflex, observations were made on the changes in the volume of

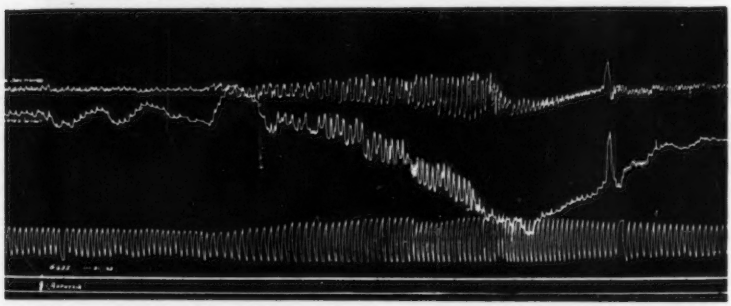

Fig. 11. The effect of rebreathing on the volume of the divided spleen. The upper tracing represents the variations in the denervated portion and immediately below the variations in the intact segment. Respiration and time are also recorded. The results obtained with the whole intact and denervated spleens are also obtained with the divided spleen preparation.

the spleen before and after denervation of the whole organ and the two halves of an organ, one of which was denervated.

The splenic reflex is abolished by section of the nerves to the spleen (fig. 8). In the experiments in which the organ was divided and one-half denervated, the innervated half decreased in volume following external stimuli while the denervated portion remained unchanged or increased slightly (fig. 9). A spleen which responds with a decrease in volume during a period of rebreathing will respond with an increase during the same procedure after being denervated (fig. 10). The divided spleen with one half denervated decreases in volume in the innervated half and increases in volume in the denervated half when the animal is allowed to rebreathe until stimulation of the vasomotor center occurs (fig. 11).

DISCUSSION

While the results of these experiments on changes in the volume of the spleen are very definite, it is quite evident that several possible sources of error must be eliminated before they can be considered of physiologic significance. The most important of these possible sources of error is the mechanical one of movements of the animal, of respiration, or of the stomach or intestines. Gross movements of the animal, as struggling, will produce marked fluctuation in the recording manometer. However, the trained animals rarely moved and the effect of such movements, when they did occur, was very evident on the record. The recording of respiratory movements in relation to the plethysmographic record of the splenic volume showed that respiration could rarely have been a factor in the changes recorded in splenic volume because typical curves were obtained in which there was no apparent change in the respiratory tracing taken at the same time. The plethysmograph was so non-compressible that movements of the diaphragm and of the thoracic and abdominal muscles could not affect it. The respiratory movements could only have been a source of error by causing a change of position of the plethysmograph. It was more difficult to eliminate absolutely the possibility that the gastric movements, by pulling on the omentum and spleen and thus causing variations in pressure in the plethysmograph, were responsible for some of the records interpreted as due to changes in the volume of the spleen. This possible source of error was controlled somewhat by section of the gastrosplenic omentum and dividing the vasa brevia completely from the stomach. Typical records were still produced after such a procedure. We were never able to eliminate movements of the stomach as a source of error from consideration, and due cognizance must be taken of it, particularly in regard to the effect of the different foods. However, in most of our observations, movements of the stomach were not a source of error and the various control experiments, as those of denervation of half the divided spleen, and those already described, definitely proved that the spleen was solely responsible for the records obtained.

A study of our plethysmographic records shows that the spleen is almost constantly undergoing rhythmic changes in volume. The volume seldom remains the same for any long period of time. There are two possible mechanisms, one or both of which produce this change in volume; contraction of the intrinsic muscle of the spleen and vasomotor activity. We have not been able to determine definitely which of these is responsible. While it is known that the intrinsic muscles of the spleen in the dog can greatly decrease the size of the organ it does not seem that these are the motor source of the rhythmic change in size. We attempted to decide this point

by a study of other species of animals in which the intrinsic muscle content of the spleen is very sparse. However, none of such animals was suitable for this work. If these rhythmic changes in the volume of the spleen are vasomotor in character, they are not dependent on general blood pressure because, when the two are recorded together no relationship can be shown. It seems, however, that the changes are due to a specific vasomotor activity of the spleen and represent variations in the amount of blood in the organ. That the intrinsic muscular system of the spleen may be of importance with regard to the mechanism of change of splenic volume cannot be ignored. Barcroft (1925) and his co-workers, in their interesting investigations in the delayed saturation of the hemoglobin in the spleen with carbon monoxid and the spleen as a source of hemoglobin, attributed their results to the action of the intrinsic muscles.

Our observations show clearly that the rhythmic changes in the volume of the spleen are altered by activity of the gastro-intestinal tract. Furthermore, the various diets affect these changes in volume differently. However, the degree to which the splenic volume is affected by digestion and the various diets as well as the physiologic significance of this relationship, is not definite. Only a larger series of experiments, with control observations on the changes in the volume of other organs, such as the kidney and lobe of liver, during digestion, would possibly establish the significance of this interesting observation.

The employment of drugs in these experiments was mainly for the purpose of controlling our method of investigation. However, the results prove that the action of such drugs is the same on the volume of the spleen in the intact animal as in the anesthetized animal. This would seem to add to the value of the results of pharmacologic studies in acute experiments.

Considerable time was devoted to the study of the splenic reflex in an effort to determine its cause and the factors involved. It seemed that it might be due to: 1, movements of the animal; 2, a change in general blood pressure affecting the spleen secondarily; 3, the action of epinephrin secreted during the emotional stress; 4, a vasomotor action, and 5, a combination of two or more of these factors.

The first consideration that the reflex was due to movement of the animal was most difficult to eliminate. However, as previously stated, the movements of the trained animals were often slight and when movements did occur the respiratory record showed it. The real proof that bodily movement was not the cause of the splenic reflex was the results obtained after denervation. When a spleen which had responded even very markedly to certain stimuli was denervated, no response occurred. The mechanical position of the spleen, plethysmograph and other factors were not changed by the denervation. The divided spleen with one-half

denervated and the nerves of the other half intact also proved this, because while both should have been affected equally if the decrease were due to mechanical factors, only when the nerves were intact did a decrease of volume occur.

That the decrease in splenic volume was not due to changes in general blood pressure was proved by recording carotid blood pressure and splenic volume at the same time. Although there were some changes in general blood pressure, they were not great enough, rapid enough, nor did they occur in close enough sequence to account for the decrease in splenic volume.

That the decrease in splenic volume was not caused by the vasoconstrictor action of epinephrin was proved by the quickness of the reaction, and conclusively by the failure of the denervated spleen or half of spleen to respond. In this connection, however, we can record one result which may be due to epinephrin. At a certain stage of rebreathing, when the vasomotor center has been very strongly stimulated, and more often shortly after fresh air has been admitted to the rebreathing apparatus, the denervated portion of the spleen will also quite frequently show a decrease in volume. We have considered this as possibly due to the action of epinephrin, although the changes of general blood pressure cannot be ignored.

The splenic reflex does not occur when the nerves to the spleen have been destroyed. This would substantiate the view that it is due to a vasomotor reflex. However, it must not be forgotten that a nerve reflex involving the intrinsic muscles of the spleen might produce the same effect. In a few experiments in which a plethysmograph was placed around the kidney and allowed to heal in position, the effect of fright, and so forth, studied in relation to changes in renal volume, was manifested by a result similar to that shown by the spleen, but to a much less degree. In the kidney the response could only have been of a vasomotor nature.

These observations on the splenic reflex prove not only that the spleen undergoes almost continuous rhythmic changes in volume but that it is constantly responding with marked changes in volume to changes in its internal environment. It would seem that this organ is constantly changing in volume in response as well to the various external stimuli reaching the individual. This reaction, which is probably vasomotor in character, is of considerable magnitude and must have some physiologic significance.

From the standpoint of the activity of the organ itself, it would seem that the rhythmic changes are for the purpose of either filling the sinuses of the organ with blood, expelling blood from the organ, or more probably both. The organ could thus obtain a supply of blood to work with or add an elaborated product or changed blood material to the circulation. The variations in the volume of the spleen associated with digestion may simply

be an expression of heightened vasomotor activity of the entire splanchnic area; the spleen may be elaborating a product to be passed into the circulation at the time of digestive activity; the old theory that the spleen acts as a pump of the portal circulation may be correct; the spleen may be a shunt to the portal circulation and the increased vasomotor activities of the organ may allow an increased amount of blood, not laden with digestive products, to reach the liver through the portal system.

The splenic reflex, while probably of less importance than the rhythmic changes in its relation to the specific functions of the spleen, is of greater general interest. Our results prove conclusively that the spleen of the dog is constantly responding to a wide variety of external stimuli by a decrease in volume. Whether these changes are a specific function of the organ or whether the whole splanchnic region is involved has not been definitely determined. In a few instances we obtained a similar result with the kidney but to a lesser degree. The greater response of the spleen might be due to its greater vascularity. It must not be forgotten, however, that the splenic reflex may be for the specific purpose of forcing some especially elaborated splenic product into the circulation.

It would seem, however, that the splenic reflex is but a visceral vasomotor phenomenon which may include all the viscera but is especially prominent because of the vascularity and large vasomotor supply of the organ involved. If this is true it forms the basis for many speculations of both a physiologic and clinical nature. It would seem that the well established hypothesis is substantiated that in time of stress the blood supply of the central nervous system and muscles is increased by constriction of the splanchnic area.

In order to make clear the following discussion it seems best to give briefly the details of the observations on two dogs which illustrate two phases of the splenic reflex. The one animal was a large, good-natured male shepherd dog, who always responded with a marked decrease in splenic volume to each kind of stimulus the first time it was applied but never twice to the same stimulus. For instance the first time hands were clapped above his head, the splenic volume decreased almost as much as our method would record. Thereafter the clapping of hands never produced a change in the plethysmographic record even though the animal might not know what was going to happen. In a short time we had used up all our methods of stimuli on the animal and it was impossible to make the volume of the spleen decrease. The animal entered good naturedly into the game, but the spleen only underwent the characteristic rhythmic changes. In another animal exactly the reverse was found to occur. This dog was a nervous female fox terrier. She always responded with a marked decrease in splenic volume, and to almost all kinds of external stimuli. Clapping hands together always produced a ready response, no

matter how often it was done. The animal would be perfectly motionless, yet each unusual sound would produce a splenic reflex. In many instances the animal appeared unconscious of the stimulus which produced the change in the volume of the spleen.

These two animals represent two opposite types; the first is the stable phlegmatic type whereas the second is the unstable nervous type. In the latter it would seem that the various environmental stimuli are constantly producing a reaction within the organism. Every sudden sound or movement and probably other means of stimulation are constantly producing a response in such an animal. This could well account for part of the fatigue or illness that occurs when some persons change their environment, for example, the farmer visiting the large city. In connection with the kidney it is more reasonable to accept the view held by many clinicians that albuminuria is often due to a nervous upset. Furthermore it might be assumed that in some persons these vasomotor changes might give rise to vague sensations not within their experience and thus produce some of the symptoms of the neuroses. The feeling of weakness as well as its site of localization which follows great fright could well be explained as the basis of such reflexes.

Since the completion of these experiments, all of which were performed during the summer and autumn of 1923, Barcroft (1925) and his co-workers have published an interesting and valuable series of papers on the carboxyhemoglobin penetration of the spleen. They found 1, that in the resting animal there was a greatly delayed formation of carboxyhemoglobin in the splenic pulp following the administration of coal gas; but that 2, the formation took place rapidly when the animal was excited. This is in accord with our observations on the changes in the volume of the spleen following excitement.

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THE EFFECTS OF THE MECHANICAL OBSTRUCTION OF THE HEPATIC VEINS AND PEPTONE SHOCK

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In a previous paper (1925) we described a method of mechanically constricting the hepatic veins. This method has the distinct advantage, *1*, of making it possible to control the outflow of blood from the liver with a minimum of mechanical trauma; *2*, of furnishing a means of studying the relations of the hepatic and systemic circulations with both essentially intact; and *3*, of restoring the circulation through the liver promptly at any time after it has been blocked.

Sudden complete obstruction of the hepatic veins causes an abrupt fall in arterial blood pressure and a rise in portal vein pressure, similar in all essential details to that observed in anaphylactic and peptone shock in the dog, except that the arterial pressure does not fall as low as in the severe forms of these types of shock.

This observation, together with the facts *1*, that peptone is known to induce reactions in smooth muscle (Schultz, 1912), and *2*, that, as pointed out by Simonds and Arey (1920), the hepatic veins in the dog are especially rich in smooth muscle, led us to investigate the possibility of constriction of these veins as the physiologic mechanism of peptone shock. The purpose of the experiments here reported was to determine *a*, whether constriction of the hepatic veins during peptone shock would induce a further lowering of arterial blood pressure, and *b*, whether the injection of peptone solution after complete blocking of the hepatic veins would cause any additional fall in blood pressure.

In one series of dogs a rubber tube was placed around the hepatic veins in the manner described in our previous paper, and temporarily left loose. Non-fatal shock was induced by the intravenous injection of a solution of peptone. As soon as the blood pressure had become stabilized at its low level from the effect of the peptone, the hepatic veins were completely occluded so that the escape of blood from the liver into the vena cava was blocked. This caused a slight further fall in arterial pressure not exceeding 10 mm. of mercury. The animal whose tracing is shown in figure 1 received 1 gram of peptone. This brought the arterial pressure down from

Fig. 1. Constriction of hepatic veins after induction of peptone shock.

Fig. 2. Peptone shock with hepatic veins mechanically occluded. Release of veins while blood pressure is at its lowest level.

150 to 70 mm. of mercury. It had risen to 72 when constriction was applied to the hepatic veins which further reduced the pressure to 62 mm. of mercury, or 8 mm. below the maximum fall induced by the peptone. The obstruction to the outflow from the liver was maintained for approximately one minute, during which the arterial pressure rose to 66. Upon release of the mechanical constriction the pressure rose abruptly to 134, and then gradually to 145 mm. of mercury.

In another series of dogs, a fall in arterial blood pressure was induced by mechanical obstruction of the hepatic veins. As soon as the pressure became stabilized, a solution of peptone was injected into the femoral vein. This was promptly followed by a further rapid fall in arterial pres-

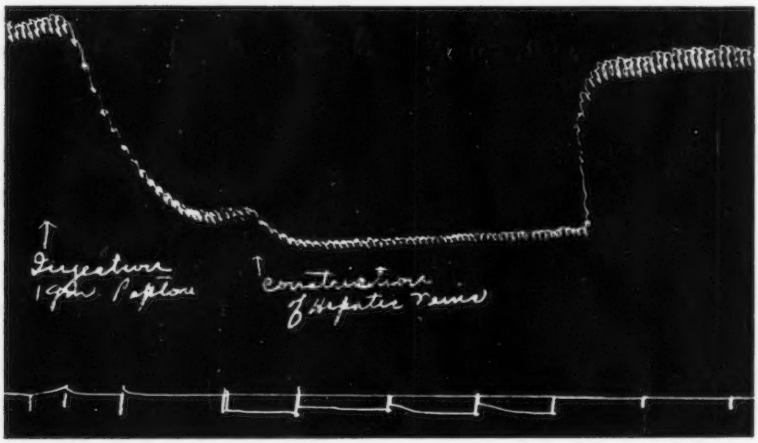

Fig. 3. Peptone shock with hepatic veins occluded. Release of veins after blood pressure had risen to the level to which it had been brought by the constriction of the veins.

sure. The results of two experiments are shown in figures 2 and 3. In the case of the first dog, figure 2, occlusion of the hepatic veins reduced the blood pressure from 145 to 88 or 90 mm. of mercury. The injection of the solution of peptone caused a further lowering of pressure to 56 mm. of mercury. This represented an additional lowering of the pressure by 36.7 per cent. The obstruction to the outflow of blood from the liver was maintained for approximately 2½ minutes during which the pressure rose to 83 mm. of mercury. Release of the constriction was followed by an abrupt rise in arterial pressure to 150 with the usual subsequent drop of some 5 mm. of mercury.

Occlusion of the hepatic veins of the dog whose tracing is shown in figure 3 caused a fall in arterial pressure from 175 to 92 or 96 mm. of mercury.

The injection of peptone caused a further fall to 58 or 60 mm., or 36.1 per cent. The hepatic veins were released after the lapse of one-half a minute and the blood pressure rose very abruptly to 105 and quickly subsided to 98 mm. of mercury, i.e., to approximately the level to which it had been brought by the constriction of the hepatic veins. During the succeeding 2½ minutes it gradually rose to 164 mm. of mercury. The fall in blood pressure following the intravenous injection of peptone into dogs whose hepatic veins are occluded is due to peripheral dilatation. Simonds and Ranson (1923) by injecting a solution of peptone into the artery to one of the hind legs, demonstrated by means of plethysmographic tracings a very marked, prompt peripheral dilatation.

Porter (1907–08), (1914) has emphasized the importance of the "percentile" change in blood pressure. It is significant that in both of the above tracings the additional percentile fall in pressure induced by the intravenous injection of peptone after obstruction of the hepatic veins, was almost exactly the same, namely, 35.7 and 36.1, respectively.

SUMMARY

1. Blocking the hepatic veins during non-fatal peptone shock caused a slight additional fall in arterial blood pressure of approximately 10 to 12 per cent.

2. The injection of a non-fatal dose of peptone while the hepatic veins were mechanically occluded caused a further rapid fall in arterial pressure of approximately 36 per cent in the dogs used.

3. This additional lowering of blood pressure was due to peripheral dilatation induced by the peptone.

4. When the constriction of the hepatic veins was maintained until the arterial pressure had risen to the level to which it had been brought by the constriction, release of the veins was followed by a prompt rise to normal.

5. When the hepatic veins were released before the arterial pressure had begun to rise, it rose abruptly to approximately the level to which constriction of the veins had brought it and then slowly returned to normal.

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STUDIES IN VIGOR

V. The Comparative Activity of Male and Female Albino Rats

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In the course of various investigations carried out at this laboratory on the factors affecting the voluntary activity of the albino rat, it has been noted repeatedly that the general level of the activity of the males was lower than that of the females. Similar observations have been made elsewhere. Wang, Richter and Guttmacher (1925) state that the average daily activity of the male varies between two and eight thousand revolutions, while that of the female varies between six and twelve thousand. Wang (1923) and Slonaker (1925) have both noted that the activity of the female is subject to a cyclic rhythm. By the use of the vaginal smear method devised by Stockard (1917) and first used on the rat by Long and Evans (1922), these observers were able to show that the peaks in the activity of the female rat correspond to the period of oestrus, while the time of lessened activity between the high points is synchronous with the period of dioestrus. No such cyclic rhythm has been noted in the activity of the male though considerable variation from day to day is characteristic.

In view of the large amount of work that is being done at the present time on the activity of the white rat, it seems desirable to make a more careful and detailed comparison of the activity of the male and female rat than has yet been made. During the past eight months upwards of two hundred animals kept in revolving wheels have been under daily observation at this laboratory according to the technique already described by Durrant (1924), (1925) and Hoskins (1925). From among these, thirty-five males and thirty-six females were selected for comparison. Of this number twelve males and thirteen females were litter controls. In connection with this point it may be well to state here that work done at this laboratory has shown that while litter controls may be desirable, they are by no means essential, since a greater variation is often found between two rats that are members of the same litter than between another two that are members of different litters. These thirty-five males and thirty-six females had all been placed in the revolving wheels

between December 24, 1924, and January 7, 1925. The average age was about forty-five days at this time, the range in age being approximately the same for both males and females (about thirty days). The males and females were kept in separate rooms under conditions as similar as possible.

The activity of both males and females expressed in revolutions per day (each revolution being approximately equal to one linear yard) averaged for each month is shown in table 1. The average for the entire six months' period was as follows: males 4138 revolutions per day, and females 7384 revolutions per day. Thus the activity of the males was only 56 per cent of that of the females. The most active male in the group averaged 8340 revolutions per day for the six months, while the least active individual averaged only 188 revolutions per day. In all there were three males whose averages were below one thousand revolutions per

TABLE 1

	MONTH						AVERAGE
	January	February	March	April	May	June	
Average age of rats in days.....	60-90	90-120	120-150	150-180	180-210	210-240	
Activity of males in revolutions* per day.....	2,984	5,302	4,630	4,682	3,993	3,145	4,138
Activity of females in revolutions per day.....	5,963	9,217	8,106	7,675	6,914	6,291	7,384
Activity of males expressed as per cent of activity of females.....	50%	57.5%	57.1%	61.0%	57.8%	50%	56%

* The revolving cylinders are one foot in diameter.

day. On the other hand, there were ten males in the group that averaged more than six thousand revolutions per day. In the group of females there were three individuals whose averages were below one thousand revolutions per day and twenty-four whose averages were above six thousand revolutions per day. The most active female made an average of 12,214 revolutions per day, while the least active made an average of 272 revolutions per day. It will be noted from table 1 that the males approach most nearly to the females in the month of March, their third month in the wheels, in which their activity is 61 per cent of that of the females. Both males and females reach their high point in the second month in the wheels, at the age of 90 to 120 days. This high point for the males is 5302 revolutions per day, while for the females it is 9217 revolutions per day. After reaching this peak in activity, which probably occurs a short time after the attainment of puberty, the average activity for both male and female groups decreases. The activity of the males,

however, falls off more slowly at first than that of the females. In April, the fourth month in the wheels, the activity of the males had dropped only 11.7 per cent while that of the females had dropped 16.7 per cent. However, by the end of June, the sixth month in the wheels, the activity of the males had dropped 40.7 per cent from its maximum while that of the females had fallen only 31.7 per cent.

A number of the animals showed a marked rise in activity with the coming of spring. This is shown in figure 1 by a rise in the average activity of the males in the month of March, and by a very much smaller drop in the average activity of the females in this month than occurred in any other month after the peak of the activity was reached. Since the

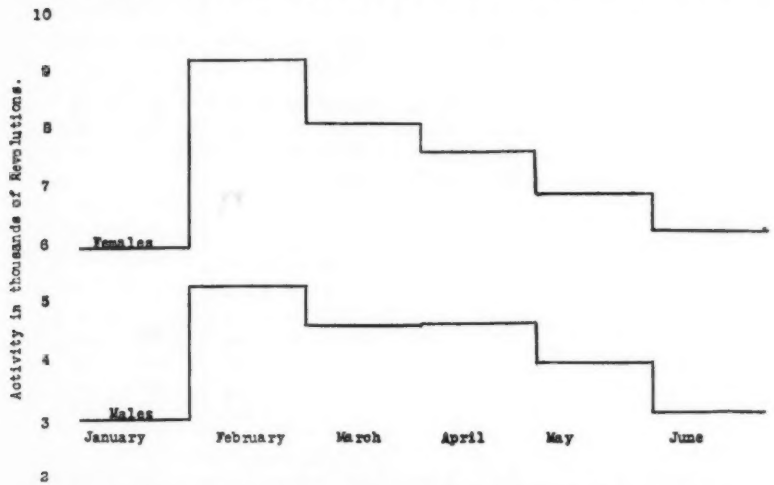

Fig. 1. Graphs of the average daily activity of 35 male and 36 female white rats for a period of six months. The average age of the rats was 30 days on January 1. Each revolution is approximately equivalent to one linear yard.

windows of the colony rooms were always opened on pleasant days when the outside temperature was not too low, it is possible that this increased activity may have been due to such meteorological changes as occur in the spring, although this is not at all certain. The data given here would indicate that this seasonal effect, if it exists at all, is much more marked in the males than in the females. A careful study of the individual records that go to make up the averages does not, however, bear out this conclusion. Of the thirty-six females whose records are considered in this report, twenty-seven showed a marked increase in activity in the spring or early summer, while twenty-five of the thirty-five males showed a similar increase in activity. With the males this increase occurred

in March in about half of the cases, thus producing a marked effect on the graph plotted from the averages. With the females, on the other hand, this increase occurred for about equal numbers in March, April, May and June, thus being masked in the averages.

TABLE 2

	MALES				FEMALES			
	Rat number	Daily average	Average variation	Per cent variation	Rat number	Daily average	Average variation	Per cent variation
				per cent				*per cent*
Litter 1:								
Born 11/5/24	752	5,052	1,635	32	756	7,395	3,171	43
Parents 0 × 236	754	933	332	36				
Litter 2:	787	10,105	4,348	43	791	17,925	6,074	34
Born 11/18/24	788	2,135	1,189	56	792	15,238	5,166	34
Parents 522 × 457	789	4,519	3,101	69				
	790	5,369	2,909	54				
Litter 3:								
Born 11/24/24	796	1,271	871	69	797	9,872	6,473	66
Parents 116 × 457								
Litter 4:								
Born 11/27/24	805	216	146	68	806	7,880	4,620	59
Parents 242 × ?					807	237	87	35
					809	663	364	55
					810	849	417	49
Litter 5:								
Born 12/10/24	811	2,445	1,211	50	816	7,620	4,116	54
Parents 689 × 691	812	7,495	2,341	31	817	10,676	7,170	67
	813	5,274	3,018	57	818	12,275	6,864	56
					819	4,724	2,993	63
Litter 6:								
Born 12/15/24	827	9,211	3,747	41	829	9,130	3,379	37
Parents 575 × 565								

It has already been pointed out that the activity of the female is subject to a cyclic rhythm, corresponding to the oestrous cycle of the animal, which is not present in the male. Wang (1923) has shown that the activity of the female falls off 90 to 95 per cent upon spaying, while Hoskins (1925) has demonstrated a decrease of about 60 per cent following castration. It would appear, therefore, that in well-fed laboratory animals, such as those used in these experiments, the sex glands furnish the larger

part of the stimulation for voluntary activity. Since this stimulation coming from the sex glands is presumably constant in the male, but varies in the female, being strongest in the oestrous or heat periods and weakest in the periods of dioestrum, it would seem reasonable to expect a greater constancy in the activity of the male with corresponding smaller variations from day to day. In order to test the truth of this assumption, a careful study was made of the variation in activity of all animals in the series for which it was possible to select litter mate controls from the opposite sex. This series consisted of twelve males and thirteen females. The daily variation in the activity of each rat was calculated for the first sixteen days of March, this period being selected because it was the time immediately following the maximum activity of the group as a whole. The results obtained in this way are shown in table 2. It will be noted that in litters 1 and 5 the percentage variation in the activity of the females is considerably greater than in the males, while in litters 2 and 4 it is approximately 20 per cent less than in the males. In litters 3 and 6 the percentage variation is almost the same for both sexes. The average variation for all the twelve males in this series is 46 per cent of their average daily activity while for the thirteen females it is 48.5 per cent. This difference is much too small to be significant and the only conclusion that can be drawn is that the daily variation in the activity of the male rat, while following no definite cycle, is still approximately the same per cent of the average daily activity as it is in the female, that is, a little less than 50 per cent.

SUMMARY

1. A study of the comparative activity of 35 male and 36 female white rats shows that the activity of the males is only 56 per cent of that of the females. For a period of six months the average activity of the males was 4138 revolutions per day, while that of the females was 7384 revolutions per day.

2. There is some evidence of a seasonal variation in activity, taking the form of an increase in activity in the spring or early summer. Twenty-seven of the thirty-six females and twenty-five of the thirty-five males showed such an increase.

3. In spite of the fact that the activity of the females is cyclic, running parallel to the oestrous cycle of the animal, while there is no such cycle in the activity of the male, the average daily *variation* in the activity of the males is of the same order as that in the females, being a little less than 50 per cent of the average daily activity in both sexes.

I wish to acknowledge my indebtedness to Prof. R. G. Hoskins for advice and suggestions in regard to this investigation.

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ON THE SUMMATION OF CONTRACTIONS IN SKELETAL MUSCLE

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The relatively huge contractile tension[2] developed in many muscles on maximal stimulation of the motor nerve renders improbable the occurrence of maximal responses in the intact animal, and suggests that the excess of fibres in muscles above the actual requirement for tension subserve the function of delicate gradation and adjustment of sustained contractions (Fulton and Liddell, 1925b) rather than the development of unwieldy contractile stress. Prolonged contractions of striated muscle, as is well known, are made possible probably entirely[3] by summation. But "in the assemblage of striped fibres composing a muscle," as Sassa and Sherrington (1920, p. 111) have noted, "the modes of summation of their contraction are of course two."

Multifibre summation. At constant initial length the mechanical tension developed in a parallel-fibred muscle in response to a single stimulus varies closely if not directly with the number of muscle units stimulated (Watts, 1924) since the "individual mechanical tensions of the several component fibres of the muscle sum additively" (Sassa and Sherrington, 1920). This type of summation Sassa and Sherrington have called "fibre" summation, or as Professor Sherrington has suggested privately, it may for greater clarity be referred to as "multifibre" summation. This has its parallel in reflex physiology in spatial summation (1906) which designates the process by which reflexes (*e.g.*, scratch reflex) are elicited or enhanced

[1] Christopher Welch Scholar.

[2] Liddell and Sherrington (1923) directed attention to the large tensions developed in a motor tetanus of a cat's quadriceps, and Fulton and Liddell (16) found that as much as 40 to 50 kilos may be developed in that muscle, i.e., 25 times the weight of the cat. Doctor Gasser has very kindly communicated to me that he and Professor Dale observed tensions as great as 25 kilos in the gastrocnemius of a cat even when the conditions of the response were not completely isometric. The gastrocnemius of a 40 gm. frog may develop 1500 to 1600 grams in a maximal tetanus at 50 per second (Fulton, 1925a), i.e., nearly 40 times its own weight. Such tensions not infrequently break the tendon.

[3] Cobb (1925) has pointed out the inadequacy of the view that the sarcoplasm of a muscle is contractile, and Fulton and Liddell (1925b) have found this surmise unnecessary to account for postural tonus.

by the *addition* of spatially distant sensory units to those which have already been or are being stimulated.

Wave (tetanic) summation. When at constant initial length a "given muscle develops a tension superior to that developed in its *maximal* twitch, there must be at play, above and beyond" multifibre summation, "that other well-known mode of summation due to fusion of successive contraction-waves within the self-same individual fibres of the muscle." This Sassa and Sherrington (1920, p. 111) have designated "wave" summation. Being tetanic in nature, i.e., caused by repetitive discharge in individual neuro-muscular units, it has its reflex parallel in "temporal" summation (Sherrington, 1906) by which process certain reflexes irresponsive to single stimuli may be elicited if stimuli are repeated at definite time intervals through the *same* sensory channels.

The present communication deals chiefly with the mechanical aspects of "wave" summation, and is a continuation of an experimental analysis of the shape of the isometric twitch. A previous study (Fulton, 1925b, 1925f) (Fulton and Liddell, 1925b) revealed that the shape of a maximal isometric twitch of intact skeletal muscle when recorded by an optical lever of high natural vibration frequency has certain noteworthy features which may be summarized as follows (see fig. 1) 1, A period of rigidity before shortening shown in figure 7 A-C (1925f); 2, an abrupt and convex (upward) mechanical ascent, showing little if any of the initial S-shape so often figured, i.e., the point of inflection (see Ballif, Fulton and Liddell, 1925, fig. 7) from concavity to convexity is usually too low to be measured, provided there is no "give" of the fixed attachments of the recording system; 3, a flat plateau or crest which had previously been described by Sherrington (1921); 4, a sudden termination of the plateau, referred to as the "angle." The more perfect the physiological[4] and mechanical conditions of recording are made the more precise is the "angle" and it may therefore be regarded as a fundamental discontinuity in the response of each individual fibre. 5, A curve of relaxation which under perfect conditions (see fig. 1A of previous paper, 1925b) appears to be wholly concave upward in shape from the start. Usually however there is initially a slight nose due to inertia of the muscle, asynchronous cessation (1925g) of contraction, etc. With the exception

[4] It may be noted in connection with the physiological condition of frogs, that among those caught or kept during the winter months in which the "condition" is probably bad, many are met with in which, even though the circulation is active, the "angle" of the twitch is from the start almost completely obscured. Frogs which have been kept for several months invariably show a somewhat modified (rounded "angle") relaxation. To ensure normality of response, with precise "angles,' i.e., responses in which all the contractile elements begin and cease synchronously,—freshly caught "summer" frogs are to be recommended. The "angle" thus provides an index of the condition of the frog quite apart from fatigue.

of the "nose," these features clearly represent the course of contraction in each individual muscle fibre and are therefore of importance to a consideration of multifibre and wave summation.

Method: The procedure employed is that previously described (1925a, 1925f), (Fulton and Liddell, 1925b). Intact gastrocnemei of decerebrate frogs have been used, and also in experiments on other points with Doctors Cobb and Liddell, the rectus, soleus and gastrocnemius of decerebrate cats have been incidentally employed. Mechanical and electrical responses were recorded simultaneously throughout. The Sherrington (1921) torsion wire myograph of 1600 per sec. natural vibration frequency has rendered the mechanical record of the same order of accuracy as the electrical record. Indirect stimulation has been used throughout.

THE ANALYSIS OF DOUBLE RESPONSES. 1. *Multifibre summation.* In order to clarify the method of analysing fused maximal contractions (i.e., wave summation) a few words must be said concerning the electrical and mechanical features of multifibre summation. Let us imagine an ideal muscle with parallel fibres half of which are innervated by one nerve, A, the other half by a fellow nerve, B. When A is stimulated singly a twitch results of 100 grams tension when recorded under completely isometric conditions; similarly when B is stimulated a 100 gram twitch results. When A and B are stimulated *simultaneously and the mechanical conditions of recording remain the same throughout the whole response* the muscle would develop 200 grams. In other words, the summation would be arithmetical. Such ideal circumstances of nerve supply and conditions of recording cannot be realized experimentally. The gastrocnemius of a frog, however, is frequently supplied approximately equally by lumbar roots VIII and IX. Samojloff (1924) and the author (1925e) have observed that the sum of the sizes of electrical deflections produced by roots VIII and IX when stimulated singly are approximately equal to the size of the maximal electrical response. Owing to the diagonal disposition of the fibres and to uncontrollable mechanical alterations *during* the response of gastrocnemius the *external* tension (i.e., the tension appearing at the tendon) does not sum completely, i.e., less tension is developed when roots VIII and IX are stimulated simultaneously than is developed in their combined but separate stimulation.

Returning again to our ideal muscle of double nerve supply contracting under perfect mechanical conditions, let us suppose that nerve A is stimulated and as twitch A commences to relax nerve B is stimulated. Under such circumstances the ascending phase of twitch B would sum *algebraically* with the declining tension of twitch A. The *tendency* toward perfect algebraical summation has frequently been observed in root stimulation of gastrocnemius,[5] but probably on account of mechanical conditions result-

[5] A more complete analysis of these responses must be deferred to a later communication.

ing from diagonal disposition, *perfect* algebraical summation does not occur. A better example of multifibre algebraical summation has been observed by Ballif, Fulton and Liddell (1925). In their analysis of the inhibition spinal and decerebrate knee-jerks they often found that the ipsilateral inhibitory break-shock (Fulton and Liddell, 1925a) caused a small ipsilateral twitch contraction apparently involving different fibres from those causing the jerk. When such an ipsilateral twitch fell during the relaxation of a knee-jerk a "nose" was formed which if carefully subtracted from the curve of an unmodified knee-jerk exactly corresponded in size and shape to the single ipsilateral twitch occurring on an unmodified base line (see fig. 7 of Ballif, Fulton and Liddell, 1925).

When many muscle units are caused to respond asynchronously but in more or less regular rotational sequence (e.g., stretch reflex) a condition of sustained tension results owing to algebraical summation of the individual units. This as Fulton and Liddell (1925b) have urged is probably the condition obtaining in many forms of postural contraction (e.g., decerebrate rigidity). Multifibre summation therefore appears to be of the utmost importance for the coördination of movement and posture.

2. *Wave (tetanic) summation.* Since the algebraical nature of multifibre summation is its outstanding mechanical characteristic, analysis is relatively simple: i.e., to determine the shape of the second element of a fused response the curve of a first unmodified response is subtracted from the fused response. Wave summation, on the other hand, is *not* algebraical and consequently demands a special method of analysis. That it is not algebraical is evident from the fact noted below (see fig. 4) that the size of a second maximal response occurring at the "angle" or during the period of relaxation of a similar first response is of almost exactly the same *shape and size as the first response or any other response occurring from a similar base line* (i.e., initial length). It is practically unmodified by the declining tension, and certainly does not sum algebraically with it (fig. 4). A second maximal response, in other words, occurring during the relaxation of the first forms for itself a new base-line the level of which is determined by the instant at which the stimulus reaches the muscle, and the ensuing response is almost completely unmodified by what has gone before and absolutely unmodified by what would have occurred afterwards. In choosing a method of analysis for such responses one must, therefore, bear these facts in mind.

If a second maximal stimulus is delivered to a muscle through its nerve at various intervals after a maximal first stimulus, modifications in the shape of the second response occur, which vary in extent with the interval between the two stimuli, being less as the interval is increased. In the account which follows, the first stimulus and the first response for brevity will be referred to as "stimulus I" and "response I;" likewise "stimulus

II, III," etc., and "responses II, III," etc. By alteration of the interval between response I and II, one can investigate certain characteristics of

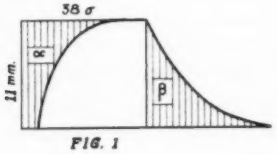

FIG. 1

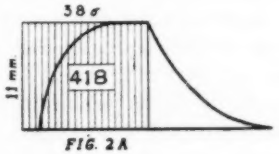

FIG. 2A

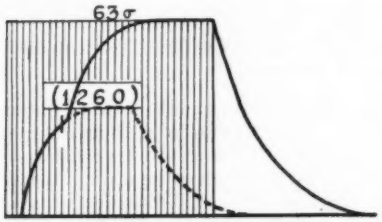

FIG. 2B

CASE I

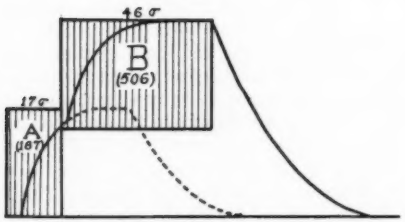

FIG. 2C.

Fig. 1. A twitch of intact gastrocnemius at 24° drawn to scale (enlarged × 1.7) to show that in a maximal twitch area α is nearly equal to area β 1 mm. vertical distance equivalent to 18 grams on original plate.

Fig. 2A. The same twitch as in figure 1. The shaded area shows the "tension-time," in this case equal to 418. The left boundary of the area is formed by a perpendicular erected from the beginning of the electrical response. The interval between it and shortening ($5\,\sigma$) represents phases 3 and 4 of the latent period (1925f). The right boundary is a perpendicular dropped to the base-line from the "angle" of the twitch.

Fig. 2B. A fused response made up of two twitches, the second of which falls during the mechanical ascent of the first. The shaded area represents the tension-time of the total response (1260).

Fig. 2C. The same as figure 1 B, but in which the tension-times of the two responses have been analysed separately as described in the text (case I).

the first response by the modifications which it is capable of producing, as it runs its course, in response II.[6] Thus when a muscle recording isometrically is stimulated during the plateau of response I, response II may last longer than response I would have lasted, and longer than a second response delivered during the period of relaxation of response I. A second response occurring during the plateau of its predecessor is therefore "augmented" by the as yet incompleted process of response I (cf. fig. 7A in previous paper (1925f) and figs. 4 and 5 below). For purposes of analysis and description, the modifications in a second response fall into four cases according as it occurs: 1, during the mechanical ascent; 2, during the plateau; 3, at the "angle," or 4, during the period of relaxation of response I. Before considering the experimental results a short account will be given of the method of analysis.

For analysis it is important to determine the area of an isometric twitch from its outline of the linear record. Since a twitch is made up of two variable components, tension and duration, the product of these two has by Hartree and Hill (1921a, p. 154), been termed the "tension-time." Since the shape of the twitch of intact muscle approaches a rectangle, much as does a calibration curve of a string galvanometer responding to a brief rectangular current (fig. 1C of a previous paper, 1925b) it is convenient to take as the area of the twitch the area of the rectangle which it approaches asymptotically. Careful examination of records, of which figure 1 is typical, proves that, in the case of the frog, this is a close approximation to the true tension-time, for area α is approximately equal to area β,— β tending, however, to be slightly greater than α. The left side of the rectangle is taken arbitrarily as the perpendicular erected from the beginning of the electrical response. At 24° the observed interval between the electrical response and the beginning of shortening is 5 to 6σ. Of this 0.3σ may be due to instrumental lag (see Fulton 1925f) and another small interval, possibly as large as 0.5 to 0.7σ (temperature experiments, unpublished) may be lost owing to the conduction time of the wave of tension which must pass up the inactive tendinous parts of the muscle to the steel hook. As these sources of error would not reduce the observed latency by more than 1σ, 5σ has been allowed for the interval between the electrical response and shortening (fig. 1). This includes a period of 'true latency' of 1 to 2σ during which the physical properties of the muscle remain apparently unaffected by stimulus, and a subsequent period of "rigidity," of 3 to 4σ when the muscle, though affected by the stimulus has not yet shortened (1925f). The distinction between these two phases of the period of latency can only be made when the muscle is relaxing (fig. 1C and 2B and C).

Modifications in the shape of the twitch may involve changes in either tension or duration. When, as in temperature alterations, only one variable (duration) is largely involved, the problem of analysis is relatively simple. But if both vary together, as when the mechanical conditions of the muscle are altered, analysis is more difficult, and comparison of the tension-times becomes necessary.

In the analysis of two, more or less fused, responses, one aims to determine the relative area of the part of response I which occurs before response II, and then to find the area of response II as modified by the remaining portion of response I.

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[6] It has been suggested that reader will be assisted in following the argument of this section if he read first the last two sections of this paper on the process of summation in which the results about to be described in detail are summarized.

Case I: When response II falls during the mechanical ascent of response I the resulting response may be almost completely fused. One example will serve as an illustration. A single isometric twitch of a frog's gastrocnemius at 24° under 40 grams initial tension has a duration, measured from the electrical response to the "angle" of 38σ; the plateau tension is 176 grams which is equivalent to 11 mm. (1 mm. = 16 gm.) movement of the shadow. For convenience the distance in millimeters is employed in making the calculations rather than its equivalent in grams. The tension-time of such a twitch is, therefore, 11×38 or 418 (fig. 2 A). Immediately this twitch was recorded, a double response was taken in which the interval between the two stimuli was 17σ. The resulting fused response might be analysed in either of two ways. One could take the tension-time of the total response,—for such a fused response tends as does a twitch, to be rectangular in shape (fig. 4A). Measured in this way the tension-time of the fused response is larger than the tension-times of two single responses. In the case above the tension-times of the fused response would be 1260 (fig. 2B) as contrasted with 836, the sum of two responses taken singly. It is clear that such an analysis does not take into account the portion of the response which was produced by stimulus I alone. If this is done, the tension-time of the fused responses is considerably smaller. Thus, one may represent the area of the portion of the response occurring before the second stimulus by the rectangular area lettered *A* in figure 2C. Its duration is the interval between the two electrical responses and its tension that which the twitch *would have developed* in the absence of a second stimulus.[7] In the case which we are considering the area *A* is 187 (11×17), while *B* is 506 (11×46). Area *B* therefore represents response II as modified by an incompleted portion of response I. In this way one can follow the successive modifications of response II as it falls at various points along response I. In passing one may note that when the fused response is thus analysed into its two component areas the sum of the two—in this case $187 + 506$, or 693—is considerably less than that of the two taken singly (836).

The staircase effect: Owing to the staircase effect a second response falling after response I has completely subsided tends to develop greater tension than the first (see fig. 2D of previous paper (1925a), and fig. 7 (1925g). To obviate this source of error, area *B* is compared with the tension-time of an uncomplicated second response, rather than with that of a first response. It has just been noted that the two components of a fused response, when added together, have a smaller area than the area of the two taken singly. If one merely doubles the area of the first response as was done above no allowance is made for treppe. If due allowance is made for this factor by adding the area of an uncomplicated second response to that of the first, the difference between this and the fused response may be even greater. In the case in point, the difference in tension-times was very small, 836 becoming only 847.

The difference in size of response I and response II due to the staircase effect and to other mechanical factors has been minimized and sometimes has been completely avoided by taking the following precautions: 1, placing the muscle before the response at a relatively high initial tension (50 to 75 grams); 2, causing the muscle to respond several times immediately before recording the response. In the responses from which figures 2 and 3 were taken, these precautions were observed and the differences between responses I and II were very slight (area of I, 407, and of II,

[7] It might be urged that the tension in this case should be that which had actually developed at the moment of the second response, but this leads to a dilemma in the case in which stimulus II follows during the latent period (as in the period of rigidity) when no tension has been developed. Using the tension which the muscle would have developed is in addition clearly in keeping with the rectangular method of analysis.

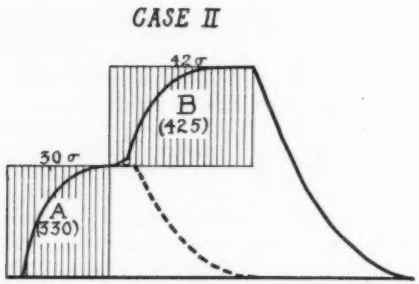

FIG. 3A

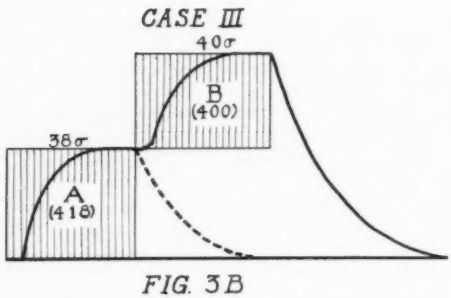

FIG. 3B

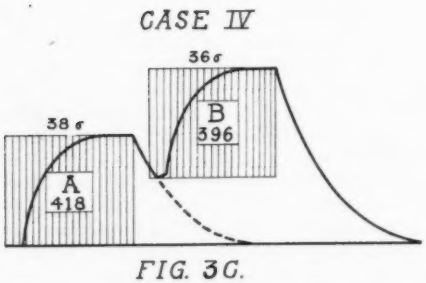

FIG. 3C.

Fig. 3A. Case II in which the second response falls during the plateau of response I. The respective tension-times and durations are indicated in the figure. It should be noted that the duration and area of response II is less than in figure 2C.

Fig. 3B. Case III in which response II falls at the "angle" of response I. Note the period of rigidity.

Fig. 3C. Case IV in which response II falls during relaxation of response I. Note the interval of 2σ ("true" latency) during which the relaxation is unchecked; following that is the period of rigidity lasting 3 to 4σ. (All of the responses plotted in figs. 2 and 3 were taken from the same preparation, a 30 gram frog at 24°C., the muscle being under 40 grams initial stretch.)

396). In general the staircase effect tends to make successive responses "higher" (i.e., greater tension) *but at the same time also briefer*. A first response taken after a prolonged rest is plotted in figure 7 of the following paper (1925g) beside the response of the same preparation, taken two minutes later, a short tetanus having been delivered between them (Cf. Adrian, 1920, p. 15). The *area* of such successive responses may or may not be the same, depending upon the initial tension, interval between the stimuli, etc. A further discussion of the "staircase" will be given in a later section.

Case II: When stimulus II falls during the plateau of a first response, analysis is relatively more simple, for response II is not complicated by the concomitant tension increase in response I. The tension-times of *A* and *B* are determined as before, according to the diagram in figure 3C.

Case III: When a stimulus II falls precisely at the "angle" of response I, response II is analysed exactly as in case II (see fig. 3c).

Case IV: When stimulus II occurs after the "angle" of response I the boundaries of area *B* are determined by the point at which the action current of response II begins, as shown in figure 3C. In this case the two phases of the period of latency are to be observed: 1, the true latency, and 2, the period of "rigidity" before shortening. These are shown in the figure.

THE INFLUENCE OF A FIRST RESPONSE UPON THE SIZE OF A SECOND WHEN THE INTERVAL BETWEEN THE TWO IS VARIED. For an experimental analysis of the influence of a first response upon the size of a second following at a brief interval it is essential that the same preparation be used in the analysis of all four cases. The numerical data illustrating these cases (figs. 2 and 3) have therefore been taken from one experiment. The results of this experiment have been confirmed in fifteen similar experiments, and may therefore be regarded as representative. Precautions have been taken to maintain a preparation during an experiment under as nearly as possible constant mechanical and physiological conditions (initial tension, temperature, circulation, etc.). The successive stimuli used were break induction shocks from two similar coils whose secondaries were connected in parallel across the same pair of electrodes.

Case I: A second stimulus delivered during the ascent of a twitch causes the ascent to become steeper and higher and, as one would expect, the duration to the "angle" of such a fused response is greater than the corresponding duration of the twitch. If the tension-time of response II is measured when it falls at successive points during the ascent of response I it is found to be largest when the interval between the two stimuli is from 6 to 10σ, varying somewhat in different preparations. But as the interval between the two stimuli is increased the tension-time of response II progressively diminishes. In one experiment (18°) at the following successive intervals between stimulus I and II: 6, 9, 15, 21, 31 and 35σ, the respective tension-times of response II were: 1167, 1065, 957, 896, 769 and 736. Concordant series have been obtained from similar analyses in other experiments. In some, however, at relatively brief intervals, the tension-time of response II at first increases slightly and then decreases

rapidly as in the above series. In one (15°) at 5, 10 and 23σ intervals, the areas were 712, 850 and 680, etc., respectively. This suggests that at very brief intervals response II is subnormal (as is indicated also by the electric response—but the diminished size of response II is of course due in large measure to lag of the string). With this exception, however, all experiments have shown that response II decreases progressively as the interval is increased. The shortest interval observed between two successive action currents in a summed response of a muscle at 20° was 3.6σ.

Cases II and III: When stimulus II occurs at successive points during the plateau of response I, there is similarly a progressive diminution in the tension-time of response II. The diminution is, however, more slight than during the ascent, and at high temperatures (24 to 25°) may even be difficult to demonstrate. At 15°, however, relatively large alterations in the *duration* of response II are regularly observed according as it occurs at the beginning of the plateau of response I or near the "angle." In the experiment mentioned in case I above at intervals of 65σ and 66σ, the area of the second responses were 710 and 692 respectively. In an-

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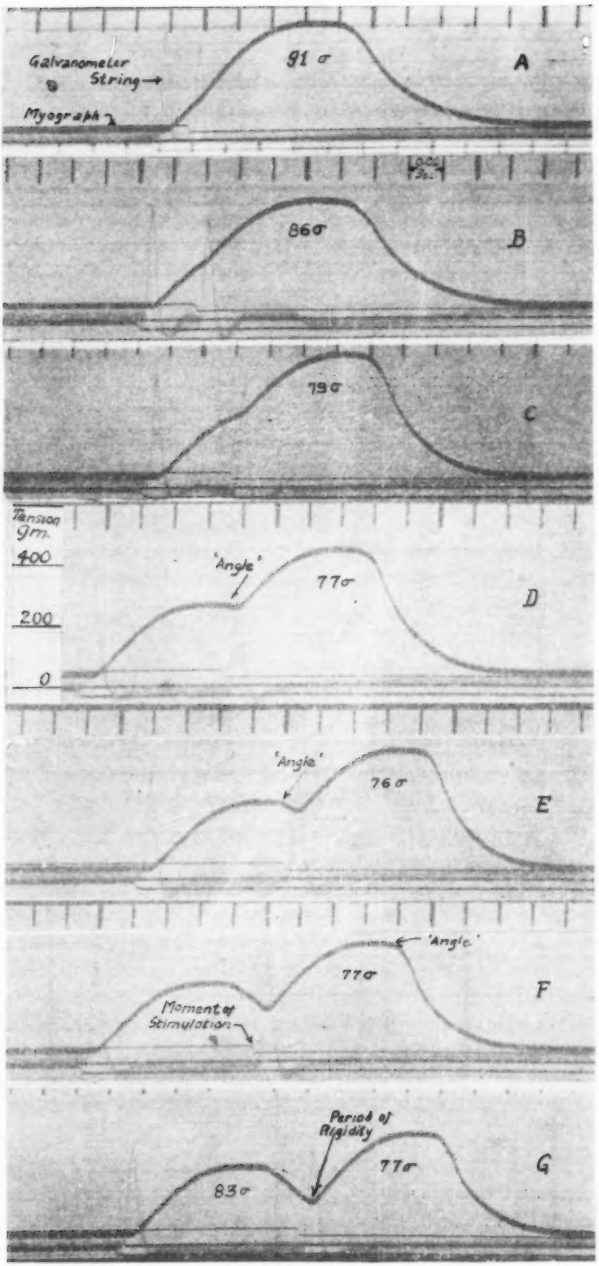
Fig. 4. A series of double responses (maximal) of an *intact* gastrocnemius of a decerebrate "spring" frog at 14° in which the second response has been made to occur at varying time-intervals after the first response; mechanical and electrical records taken simultaneously. The stimuli, which were delivered to the nerve, were maximal *break*-induction shocks of two coreless coils the secondaries of which were connected in parallel across the same pair of electrodes. The exact moment of stimulation for each response is indicated on the records by special magnetic signals which cast shadows above and below the line of zero tension. Time signals at the top of each record indicate 0.02 second. String tension: 5 mm. per millivolt at magnification of 295; diameter of string 2.5 μ; 8,000 ohms shunt across galvanometer terminals. The galvanometer leads were two fine silver chloride pins (0.4 mm. in diameter) one inserted into the belly of the muscle the other into the tendon *distal* to the point of attachment of the tendon to the myograph. Natural vibration frequency of myograph when unattached, 1,600 per second; 5.5 mm. vertical movement of the myograph = 100 grams tension. Magnification of tendon movement × 40.

Duration of unmodified first response 83 σ, and tension-time 954 (tension 210 grams); duration of unmodified 2nd response following on first within 0.1 to 10 seconds 77 to 78 σ; tension-time 930 (tension 220 grams). The difference between the two is caused by the staircase effect. The following table gives the analysis of figures A–G. The second response in D occurs 1 σ after the "angle" of response I.

	Interval between I and II responses (*0.001 sec.*)	Duration of second response (*0.001 sec.*)	Tension–time of second response ($\sigma \times mm.$)
A	19	91	1365
B	33	86	1070
C	53	79	908
D	84	77	824
E	89	76	836
F	103	77	860
G	110	77	924


Galvanometer String
Myograph
91 σ
A
86σ
B
79σ
C
Tension gm.
400
200
0
'Angle'
77σ
D
'Angle'
76σ
E
'Angle'
77σ
F
Moment of Stimulation
Period of Rigidity
83σ
77σ
G

other experiment at 12.5° with an interval of 60, response II lasted 78σ and at 67σ interval, it lasted but 71σ. The duration of response I was in this case 68σ. There is seldom any measurable difference in the *tension* developed by response II wherever it occurs in the plateau. The chief form of augmentation during this period is, therefore, one of enhanced duration. In the example last cited response II occurred within 1σ of the "angle" of response I. As this is as accurately as it can be timed, this may be taken as an example of case III. It is worthy of note that the duration of response II when at the "angle" is nearly that of response I, and their respective areas closely approximate to one another.

Case IV: During the relaxation period from response I of an unfatigued muscle, i.e., at any point *after* the "angle," *the duration of response II tends to be constant.* In other words, a first response is incapable during its relaxation period of producing any modification in the duration of a second response. In one experiment at 21° in which 6 determinations were made during the descending limb at the following intervals after the "angle:" 5, 7, 9, 21, 29, 47σ, the respective durations were: 43, 48, 48, 49, 49, and 48σ; this has been confirmed in a large series of observations both low and at high temperatures. The tension developed at successive points along the descending limb tends to grow slightly larger as the rate of fall diminishes, but the *shape* is always the same and there is nothing to suggest algebraical summation. In the experiment just cited, at 7σ (at the point of most rapid fall) the "height" of response II was 7.6 mm. At a point 47σ from the "angle" (base line) it was 8.0 mm. This makes the tension-time of response II *increase* slightly during the descending limb of response I, an effect which may be attributed in part at least to the necessity of counteracting the rapidly falling tension in the early stages of relaxation. *In any case it is clear from these observations that the "angle" of the first response marks the point at which the power of augmenting the duration of a second response ceases.* Photographic records illustrating the four cases are shown in figure 4 A-G. In figure 5 a similar series of two responses is shown of the soleus of a cat. Here the same type of augmentation is observed, and being a red muscle which responds slowly it is of interest to see the degree of augmentation is even more marked than in white muscle (see legend to fig. 5).

Fatigue: In the account just given of the four cases of summation, the absence of fatigue has been assumed. If, however, the circulation is stopped or the muscle is excised the "angles" lose their precise nature and the first part of the descent becomes convex in place of its normal upward concavity. It has been shown that this is one of the early signs of fatigue (Fulton, 1925b), caused by an incomplete and possibly asynchronous cessation of contractile activity. Though analysis (owing to the absence of the "angle"), in these responses is less accurate, it is nevertheless clear

that second responses occurring after the "angle" in the convex position of the descent tend to be somewhat longer than when they occur later in the concave position. Thus in a moderately fatigued muscle at 15° in which the convexity had considerably obscured the "angle" response II when it occurred 6σ after the "angle" was 64σ in duration, and when it occurred 20σ after it, was but 61σ in duration. This may be seen even more markedly when a twitch is put in at various points during the relaxation from a tetanus.

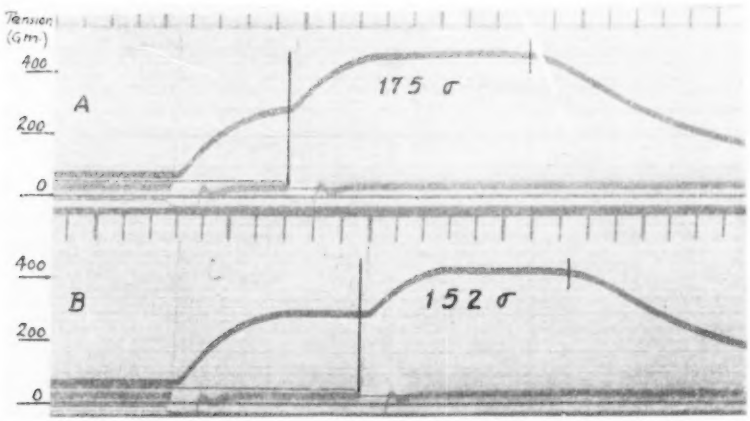


Fig. 5. Two double responses of the intact *soleus* of decerebrate cat taken at different intervals. Conditions as in figure 4 (including tendon movement and tension scale). Temperature of muscle = 35.

A. Interval 83 σ Duration of second response 175 σ.

B. Interval 140 σ Duration of second response 152 σ. In another response of this preparation (not reproduced) with an interval of 20 σ the duration of response II was 210 σ. Being a red muscle the prolonged flat plateau is noteworthy. Note behavior of the interval between the electrical and mechanical response in B and compare with A (especially in the 2nd response).

Relation of response I to response II. When the interval between stimulus I and II is of the order of 3 to 15 or 20σ the resulting response is completely fused at 20° and its shape is that of a large twitch (fig. 4). If a series of such completely fused responses are analysed according to the method indicated in figure 2B, one finds that the tension-time of the summed response progressively *increases* as the interval between stimulus I and II becomes greater. Thus at 14° at intervals of 10, 13 and 20σ the total tension-times were respectively 1493 (13.7 × 109), 1554 (14.0 × 111) and 1702 (14.8 × 115). The increase in tension developed with increasing interval has been observed in the past, notably by Adrian and Lucas (1912),

while the increased duration one would anticipate from the greater interval between the stimuli. However, it will be remembered that the duration of response II, when analysed separately, as in figure 2C, *decreases* with increasing intervals. It follows from these two facts that the degree of augmentation produced by response I on response II does not vary *directly* with the interval between them. Suppose response I to be of 60σ duration to the "angle," and a stimulus II falls at the end of 20σ (i.e., during the ascent of I), response II will *not* be increased to 100σ as a result of the 40σ remaining to response I. The augmentation in duration (and in area) would in fact be only 20 to 25 per cent of this, i.e., not more than 8 to 10σ. and the response would be only 68 to 70σ in duration. This means that the muscle acts less and less economically as the interval between the two stimuli is decreased, which is in harmony with A. V. Hill's (1922) observation that the efficiency of muscular contraction decreases with the speed with which it is performed.

The bearing of this upon the optimal interval for stimulation is made clear by a comparison of areas *A* and *B* in figures 2C to 3C. In figure 2C the sum of areas *A* and *B* is 693 (187 + 506), and here the interval between the stimuli is 17σ. In figure 3A with a 30σ interval the sum of the two is 755 (330 + 425), and finally when response II begins at the "angle" of response I the sum of the two areas is 818 (418 + 400). With the exception of responses in which the staircase effect is very marked, the sum of the two responses always diminishes again when response II falls after the "angle" of I. In this case the sum of the two is 814 (418 + 396), as shown in figure 3C. If duration is considered rather than the area, there is invariably a diminution in the sum of the two when the second falls after the "angle." In this case 78σ (38 + 40) becomes 74σ (38 + 36) after the "angle."

THE GENESIS OF TETANUS. From what has just been said it follows that the most economical rhythm at which to generate a tetanic response varies with the duration of the twitch—measured to its angle. The average duration of a twitch of a frog's gastrocnemius at 24°[8] and at moderate initial tension (30 to 40 grams) is 37 to 40σ. It follows from this that a frequency of 25 to 30 per second would give rise to a response in which each successive stimulus would fall at or just before the "angle" of the preceding response. A series of such responses (at 12.5°) is shown in figure 6. In the first, A, the initial tension was negligible, so that the increased size of response II above that of I is marked. It is worthy of note in this series

[8] The frog's gastrocnemius at 24° has been used as in example here and in figures 2 and 3 since at this temperature the responses of frogs' striated muscle are of almost exactly the same shape and duration (*i e.*, 36–40σ for the twitch, Ballif, Fulton and Liddell, 1925) as that of "white" striated muscles of mammals. The latency (Fulton, 1925f) of the latter, however, tends to be slightly less.

that each successive response (save III) is shorter than the one preceding it. This is a manifestation of the fact that the duration of the terminal mechanical response of a tetanus ("after-action") of a short tetanus is invariably of shorter duration than the twitch immediately preceding the tetanus (1925c). In figure 6B the initial tension is somewhat higher

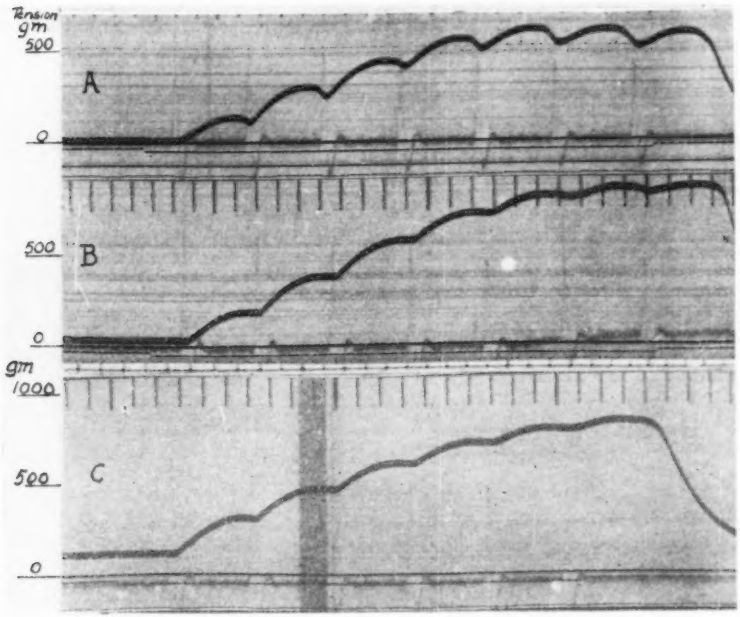


Fig. 6. Responses of the intact gastrocnemius of 35 gram winter frog at 12.5° stimulated at 14 per second. Natural frequency of myograph 1600 per sec.; diameter of string 2.5 μ at tension of 4 mm. per m.v. Time above 0.02 second 2 cm. vertical distance equivalent to 600 grams tension, other conditions as in figure 4.

A. Muscle slack; initial tension 5 grams.

B. Muscle at 30 grams initial tension.

C. Muscle at 120 grams initial tension.

Note the increasing duration of the individual responses as the initial tension is raised.

(30 grams) with the result that the successive responses are more perfectly fused. The electrical responses are also somewhat larger. In figure 6C in which the initial tension is still higher (110 grams) each successive response falls a few sigmata before the "angle" of its predecessor.

Increasing the rate of stimulation increases the rate of ascent, but the tension of the plateau when reached is but little increased by the high rate

of stimulation. In figure 15C the plateau is nearly reached at the end of the response, which is 420σ in duration. The same preparation when stimulated at double the rate reached its plateau is 280σ but its plateau tension was only 5 per cent higher.

Figure 7A-C shows the responses of the same preparation as that from which figure 6 was taken, but at a higher temperature (18°); the rate of stimulation was the same. In the first response the muscle was slack so

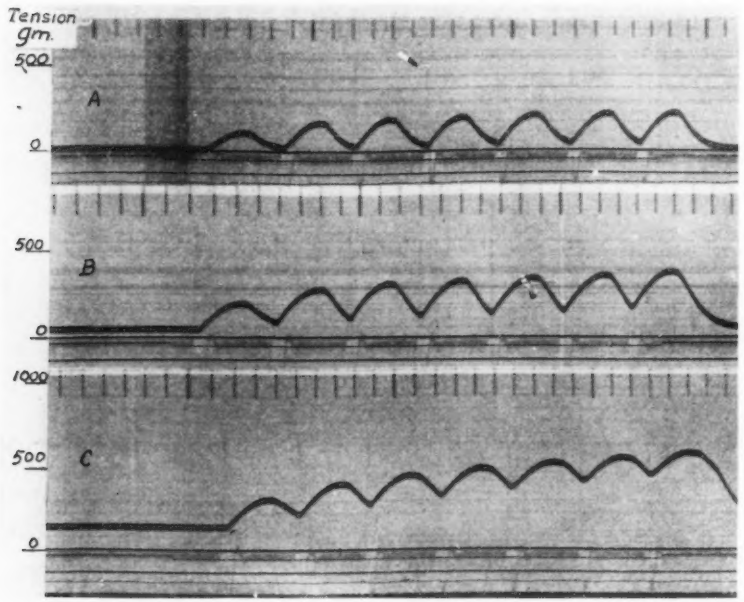


Fig. 7. From the same preparation as the record shown in figure 6 but at 18.0° instead of 12.6°. Conditions otherwise the same.

A. Muscle initially at low tension (3 to 5 grams).

B. Muscle at 25 grams initial tension. Note increase in duration of the individual responses as compared with those of A.

C. Muscle at 120 grams initial tension. Note the initial convexity of the relaxation in the terminal response.

that the "staircase" effect is very marked. It may be noted that even though the successive responses are almost completely discrete, the successive electrical responses show considerable diminution in size. The successive diminution in the action currents is, however, more marked in figure 7C in which the successive contractions are more fused owing

to the increased initial tension. These responses were taken from winter frogs and the ease with which they are fatigued is illustrated in 7C in which the terminal response of the tetanus shows considerable convexity as contrasted with those of A and B (see on fatigue above, p. 222).

Records such as those reproduced in figure 6 admit of accurate analysis of the individual components of the ascent. Since each successive response occurs but a few sigmata before the angle, little or no augmentation occurs from one to the next. As the interval is exactly the same between each stimulus, the duration is a constant and the only variable to be analysed is tension. Thus in figure 6, which is made up of 7 responses, the successive increments of tension are (in grams): 165 (24.3), 222 (21.5), 210 (20.5), 160 (19.0), 105 (18.0), 60 (17.0), 15 (16.7). This is a total of 935 grams. The corresponding electrical responses are given in parenthesis. It will be noted that responses II and III are larger than I. This is true when the initial tension is small. At higher initial tensions as in figure 6C (120 grams) the successive increments are (in grams) 201, 192, 180, 138, 90, 45, or a total of 845. This response had not reached its plateau, but it is significant that each successive increment is smaller than the previous one.

THE "STAIRCASE" AND THE SUPERNORMAL PHASE. The "angle" and the rectangular method of analysis have made possible a reconsideration of the question raised by Hartree and Hill (1921b, p. 406) as to whether there exists in the period of recovery of muscle a phase of excitability supernormal contractility comparable to the supernormal phase in nerve investigated by Adrian (1920, 1921). Such a phase of contraction must be carefully distinguished from the staircase effect. That the latter is not due necessarily to fatigue, or to over-shooting of the lever (Adrian, 1920, p. 15; Fröhlich, 1905) is evident from figure 7A above (cf. Gunzberg, 1918; Gruber, 1923). Increased blood flow which has been studied by Gruber (1924) can also be ruled out as an explanation of the "staircase" since it may occur after an interval as brief as 40σ (see fig. 6), which is far too short a time for an appreciable alteration of the blood supply. The effect, therefore, is a genuine increase in the response, and that it has nothing to do with a supernormal phase is evident from the fact that the effect has often shown itself in the present experiments at intervals as great as 30 seconds to a minute between stimuli (cf. Hartree and Hill, 1921b, p. 409). The "staircase" also is much less marked in excised than in intact muscles.

Adrian (1921, p. 215) has demonstrated that the supernormal phase of nerve and cardiac muscle is not associated with an increased action current, and this result has been confirmed in the present investigation. Hartree and Hill (1921b) found in some of their experiments what appeared to be a supernormal phase of heat production, but it did not always occur, even

at a low pH and they were unable to discover the cause[9] of the irregularity. These authors, assuming algebraical summation and the absence of the "staircase," also attempted to demonstrate the existence of a supernormal mechanical response in skeletal muscle. They found that when the response II followed during the relaxation of I, or after I had just subsided, response II was larger than I, and this they regarded as evidence of a supernormal phase. But since as we have seen "wave" summation is *not* algebraical, the algebraical method of analysis does not apply. When the curves of Hartree and Hill are analysed by the rectangular method (estimating the position of the "angle") the evidence for a supernormal phase disappears. Moreover, as we have just seen above, the duration of response II is the same at any interval after the "angle" of I up to at least one minute, even though II may be itself somewhat larger than I. There is in other words no progressive diminution of the size of response II such as would be necessary if a supernormal phase existed similar to that in nerve. It seems evident, therefore, that in the responses of intact muscle there is no satisfactory evidence of a period of supernormal contractility.

The view put forward by Mines (1913) and others to explain the "staircase" is possible, namely that an optimal hydrogen ion concentration is necessary for the contractile response and that this is achieved only after several responses have occurred, but this view has been severely criticised by Adrian (1920) on the ground that the supernormal phase (or staircase?) is best seen when a muscle is perfused at an acid pH and on Mines' hypothesis it should be most apparent with an alkaline perfusate. Another possibility suggests itself from the work of Gasser and Hill (1924). If the contractile elements of a muscle are to be regarded as an internal elastic network bathed in a viscous medium, it is then conceivable that after a rest the viscosity and frictional resistances of the muscle are large owing to "setting," while after several responses the viscosity and friction diminish and the contractile elements are more effective in producing active tension. In favour of this it may be noted that the twitch of a muscle after a prolonged rest is small but greatly prolonged (Fulton, 1925g, fig. 7; also b, figure 2D). In the same way an "isometric" response in which but 0.02 mm. tendon shortening is permitted is of greater duration (Fulton, 1925g, fig. 7) than a similar twitch in which 0.50 mm. tendon shortening is

[9] In view of the close parallelism between initial heat production and the size of the action current (1925g,h) and the fact that in responses of a muscle under appreciable initial tension to two closely timed stimuli the action current of response II is smaller than that of response I, it would be surprising if the heat production of II were regularly greater than the heat of I. When, however, a muscle is initially slack (see 1925a) and action current of response II is greater than of I, under these circumstances one would anticipate greater heat for II than I, but such an increase in heat could scarcely be regarded as proving a supernormal period of contractility.

permitted. It would thus appear that *when the contractile elements of a muscle encounter resistance to shortening whether internal or external in origin their response is more prolonged* (see following paper, 1925g).

THE PROCESS OF SUMMATION. Mines (1913) suggested that the summation of stimuli in contractile tissues is brought about by the accumulation of a local concentration of ions at contractile surfaces within the fibre. If this were strictly true a second stimulus falling at the "angle" of its predecessor would not cause further shortening, but summation of the falling tension of response I with attempted rise on the part of II would occur and the muscle would merely continue at its same length. At the end of the plateau of I when the exciting ions are probably completely exhausted a *second response begins fresh and from an entirely new base line.* Summation then cannot be regarded as an effect merely of ionic accumulation, but appears to be made possible by something more in the nature of a "catch" mechanism in which each successive response begins where its predecessor left off and from a new base line. Ionic accumulation undoubtedly occurs when a muscle is stimulated above its optimum rate, and while this makes the ascent of a tetanus slightly more rapid, it causes the muscle to act with less efficiency and induces premature fatigue.

Beck (1923) has recently pointed out that a muscle stretched at the height of tetanus attains greater tension than is developed during an isometric response whatever the initial tension be. The author concludes from this that the primary change following stimulation is one involving shortening, but that a secondary change occurs which results in increased resistance to extension. This is interpreted as due to a "catch" mechanism. The high tension attained by stretching during the tetanus plateau, however, is not maintained. This Beck attributes to fatigue but Gasser and Hill (1924, p. 435) interpret "the curve as that of the approach of an elastic viscous body to equilibrium from above."

It would in fact appear that the tension already attained in a response is largely *maintained* by the viscosity of the muscle fluids, while further increase in tension is brought about by the contractile elements. For example, when response II occurs at the "angle" of its predecessor one may infer from the resulting period of rigidity that the viscosity of the muscle is suddenly increased. This increased viscosity would serve within limits to maintain the tension already achieved. In this way the contractile elements would begin anew from a relatively low internal tension, and thus produce a full sized or nearly full sized response, without having concomitantly to maintain the tension already developed. But during the ascent of a tetanus the viscosity becomes more and more inadequate to maintain the increasing tension, with the result that more and more tension is thrown on to the contractile elements. The tension becomes finally so great that no further shortening can occur, and the result is the equilibrium of the tetanus plateau.

Such is the outline in most general terms of a view of summation which appears to be consistent with all observations at hand. This view is in reality an elaboration of the "dual-process" theory of von Kries (1880) the historical aspects of which have recently been reviewed by Gasser and Hill (1924, pp. 433–435).

THE NATURE OF THE ISOMETRIC TWITCH. It will be convenient at this point to summarize briefly the inferences which may be made from the experiments recorded in the previous pages regarding the course of contraction in a single response.

Let us consider first the significance of the "angle." The conditions under which it is observed guarantee its being a feature of the normal course of the muscular response of each constituent fibre, and since it is found in the responses of sartorius and gastrocnemius of frogs and in the rectus, gastrocnemius, soleus and tibialis of the cat, it may be regarded as a general property of responses of skeletal muscle fibres. The "angle" appears to indicate some fundamental discontinuity in the contractile process, and the possible nature of this discontinuity deserves consideration.

The properties of an unfatigued muscle during the period (of relaxation) following the "angle" have been shown by three different methods to differ from its properties during the period preceding the "angle." 1, The *rate* of fall is nearly constant at any given temperature (Fulton 1925b, c). This together with its concave shape suggests a passive process as distinguished from the active processes preceding the "angle"; 2, the temperature coefficient for 10° of the rate of decline of tension is only 1.3 to 1.4 while the coefficient of the twitch duration is 2.00 to 2.10 (1925d). This again is indicative that the declining tension is a purely passive physical process; 3, finally a first response at any point preceding its "angle" is capable of augmenting the duration of an ensuing response, but after the "angle" the power of augmentation ceases. This suggests that it is the concentration of ions responsible for contraction which ceases (or becomes abruptly inadequate) at the "angle."

The experiments just recorded were designed to investigate the course of the ionic changes during the twitch. For brevity, let the excitatory ions responsible for contraction be referred to as E-substance, or, following Sherrington's usage, simply as "E." It is desirable to know how much of "E" is present within the muscle fibres at any given instant following a stimulus. In the case of the motoneurones in the spinal cord this has been tested by the facility with which the "E" is "neutralized," i.e., inhibited at any given instant (Sherrington, 1925, p. 530). Since inhibition is impossible in the case of a peripheral twitch, the method adopted in the foregoing experiments has been to measure "E" by its power of augmenting a succeeding response. When response II follows within 5 to 10σ of the first stimulus, it is more augmented by the "E" of response I

than at any other time. The degree of augmentation diminishes very rapidly during the ascent of I, and towards the end of the plateau of I, augmentation, though clearly present at low temperatures, is sometimes difficult to demonstrate at high. Finally, after the "angle" of response I has passed, it is impossible to demonstrate in an unfatigued muscle any augmentation at all at any temperature. This leads one to the view that the "E" ions are liberated, as it were, in a burst, that they are rapidly "neutralized" during the ascent of the twitch, and finally become suddenly exhausted at the "angle." The muscle, being unable to follow these apparently abrupt ionic changes, returns to its resting shape as rapidly as its internal viscosity permits..

If this reasoning is correct, it follows that, whereas the galvanometer string analogy suggested previously (1925b) is adequate to account for the "angle" and return to the base-line, it is misleading if applied to any other part of the twitch,[10] for the ionic changes underlying the ascent of the twitch appear to rise abruptly to a maximum, subsequently falling rapidly until a temporary equilibrium is reached during the plateau. This is in keeping with Gasser and Hill's (1924) experiments in which they found a maximum resistance to a stretching force at an interval of about 5σ after the stimulus, and that this resistance rapidly diminished during the course of the response. With this in mind one will readily understand why two closely timed responses are less efficient than two more widely separated.

It is difficult, however, to understand why the ionic process should cease abruptly, for if the H ions are chemically neutralized or are disposed of by diffusion, one would anticipate that the curve representing their concentration during the twitch would descend asymtotically to the base-line. Prof. A. V. Hill has very kindly suggested to me that the "angle" probably represents an abrupt phase reversal of a heterogeneous system comparable to that which occurs in a $CaCO_3$ CaO, CO_2 system, in which as the concentration (pressure) of CO_2 is diminished a certain critical concentration is reached at which all the ionized CO_2 is converted into $CaCO_3$. During the falling concentration of H ions, if this be so, a point is reached at which the ions become abruptly combined, and this causes the "angle."

Following Gasser and Hill (17), I have assumed that the viscosity of the muscle increases as a result of stimulation. The enhanced viscosity added to the resting viscosity appears to act like a catch mechanism and in this way is of use to the muscle in facilitating the summation of contractile responses. This suggests that the shape of the isometric twitch is a resultant of two dependent variables: shortening and viscosity. This is shown diagrammatically in figure 8. The twitch according to this, results when contractile activity overcomes the viscous impediment. During

[10] These considerations likewise render untenable the assumption of a rectangular fundamental process (1925b).

the first 5σ of the twitch the viscosity is represented as equal to the contractile tension. The plateau is shown in this diagram by an interval during which the falling ionic concentration is compensated for by falling viscosity, the resultant of which is a flat plateau. The shape of the ionic curve in the figure has been inferred from the experiments on augmentation, but the shape of the viscous curve is postulated. It is known, however, that the viscosity is high at the beginning and that it rapidly diminishes during the response, becoming at the "angle," equal or nearly equal to the resting viscosity. The evidence for the last statement comes from the fact that a second response following at any point after the "angle" is of nearly constant size both in amplitude and duration. The slightly smaller amplitude of a second response at a short interval after the "angle" may indicate a residuum of viscosity above the resting value, or it may be

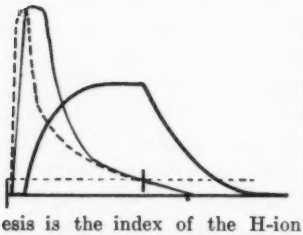


Fig. 8. A diagrammatic representation of the "fundamental" processes in the twitch as inferred from the experiments on augmentation of second responses. The first vertical line on the left indicates the moment of the beginning of the electrical response. The heavy line gives the actual shape of the twitch, while the smooth line, *C*, rising abruptly indicates the course of the internal tension development and this by hypothesis is the index of the H-ion concentration at the contractile surfaces. The dotted line represents the increase above the resting value of the internal viscosity of the muscle following the stimulus. The course of this curve is more uncertain, but to account for the period of rigidity it is made to rise more rapidly than the ionic curve for the first 5 sigmata, and it then falls more rapidly than the ionic curve to permit shortening. The dotted horizontal line is the possible threshold of the contractile elements, or according to Professor Hill's suggestion the concentration of H-ions at which an abrupt phase reversal occurs.

accounted for by the necessity of counteracting the rapidly falling tension during this period, as suggested above.

Mathematical analysis of the twitch made in these lines has been so far unsuccessful. However it is clear that the equation would be one of the general type put forward by A. V. Hill (1922) in his analysis of the effect of speed of contraction on the mechanical efficiency of human muscles.

SUMMARY

Responses involving *multifibre* summation (i.e., the additive effect of increasing the number of muscle units involved in a response), being algebraical are readily analyzed. *Wave* summation (i.e., the effect of successive responses (waves of contraction) in the same fibre or fibre group) is not algebraical in nature and therefore requires a special method of analysis which is described. The present communication is chiefly

concerned with the electrical and mechanical aspects of "wave" summation. The course of contraction in the isometric twitch has been investigated by studying the modifications which a first (maximal) response is capable of producing in a second when the second occurs at various points during the first. Modifications in a second response are divided into four cases according as they occur: 1, during the mechanical ascent; 2, during the plateau; 3, at the "angle;" 4, during relaxation of a first response.

1. There occurs a progressive diminution in the area ("tension-time") and duration of a second response as the interval is varied between a point 5 to 10σ after the beginning of response I and the "angle" of response I. The power of augmenting the duration of a second response is, therefore, greatest at a point 5 to 10σ after the stimulus of response I. The power of augmentation diminishes rapidly during the mechanical ascent of a first response and it is inferred from this the activating ions are dissipated with a corresponding rapidity, dissipation being complete at the "angle."

2. After the "angle," i.e., at any point during relaxation, the muscle possesses no power of augmenting the duration of a second response. This favours the assumption that contractile activity ceases at the "angle."

3. Records are shown of tetani in which each successive stimulus falls at the "angle" of its predecessor (figs. 15 and 16). The successive steps in the ascent are analysed.

4. The mechanism of summation of stimuli is discussed, and the inadequacy of Mines' view that a local accumulation of activating ions causes summation is pointed out.

5. It is concluded that a genuine staircase effect exists and that it may be accounted for by diminishing viscosity of the muscle with successive responses. No adequate evidence was found for the existence of a supernormal phase of contractility. The significance of the "angle" in the isometric is discussed.

I wish to express my thanks to Sir Charles S. Sherrington for his encouragement and for invaluable advice and criticism during the experiments and during their preparation for publication. I am indebted to the Welch Trustees (Oxford) for defraying the considerable expense of photographic plates, to the President and Fellows of Magdalen College for making possible the purchase of the galvanometer, and to Mr. J. O'Neill for constructing the myograph, the signals and other delicate instruments used in the research.

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THE INFLUENCE OF SHORTENING ON THE SIZE OF THE ACTION CURRENT AND THE DURATION OF THE MECHANICAL RESPONSE OF SKELETAL MUSCLE

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A consequence of the oblique direction (*e.g.*, pennate arrangement, Nicolas Steno, 1667) of fibres in many muscles is that the active fibre constituents may shorten even when the muscle as a whole contracts under completely isometric conditions (*i.e.*, constant length), and the extent of the shortening varies among individual diagonally arranged fibres (Fulton, 1925e) according to their position with reference to the longitudinal axis of the muscle.

The alterations in nature of mechanical responses when varying degrees of shortening occur *during* the response, therefore deserve careful consideration.

Method. The optical isometric recording lever of high natural frequency (1600 per second) previously described (Fulton, 1925a, 1925g; Fulton and Liddell, 1925a, 1925b) was arranged in association with a string galvanometer, and simultaneous mechanical and electrical records obtained. In figure 2 the records were obtained with a myograph of 500 per second natural vibration frequency; in all others the instrument of 1600 per second was employed. Intact gastrocnemei of decerebrate frogs were utilized and the results so obtained have been incidentally confirmed in experiments (dealing with other points) performed on rectus femoris, gastrocnemius and soleus of decerebrate cats (Fulton and Liddell, 1925a, 1925b). In all cases muscles have been stimulated through their nerves by *maximal* break induction shocks.

Early experiments (Fulton, 1925b) demonstrated the importance of absolutely rigid fixation of the recording muscle, for as will be shown presently, a "give" of even 0.10 mm. on the part of the fixed attachments greatly modifies the nature of the mechanical response. The gastrocnemius (frog) was held in place by a fine drill passing through the bony parts of the knee-joint and held in a special metal upright (see fig. 2, Fulton, 1925g). The detached tendon was attached to the isometric

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lever by a light tempered steel hook which was passed beneath a tight ligature around the tendon. Care was taken to free the muscle from fascia, skin and connective tissue around the tendon. The rectus femoris of cats was attached directly to the lever by a hook passing through the edge of the patella.

Isotonic responses involving lifting of a weight which has inertia and acquires momentum, are inevitably inaccurate. Varying degrees of shortening have, therefore, been permitted by connecting the tendon to a torsion-wire myograph at various distances along the horizontal arm projecting from the axis of the myograph lever (see fig. 1; also figs. 1 A and B of previous paper, 1925g). The extent of the tendon-shortening in any given response can then be calculated from the known constants of

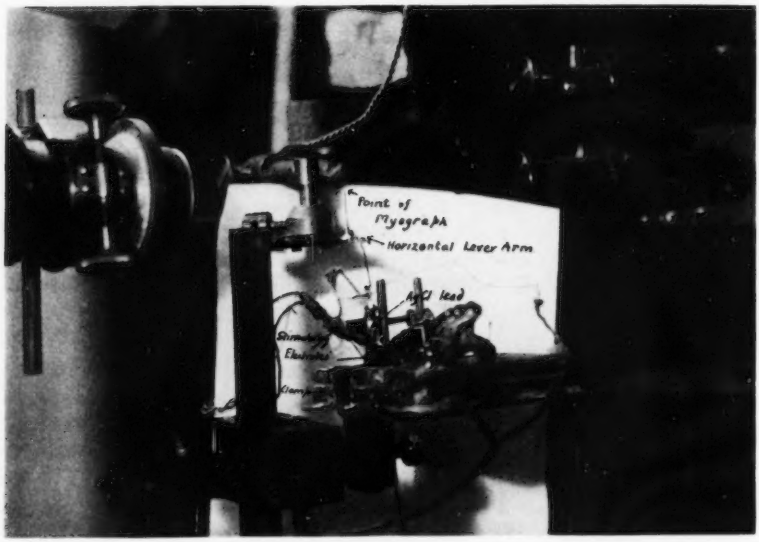

Fig. 1. "Close-up" photograph of isometric lever showing an intact gastrocnemius preparation and the horizontal lever arm to which tendon of the muscle could be attached at varying distances from the axis of the torsion wire. The fine tip (0.06 mm. in diameter) of the myograph needle cannot be seen in the reproduction. The galvanometer can be seen in the background and the focussing lens at the left. The stimulating electrodes are on the nerve, and the "wick" of the tendon galvanometer lead can be seen resting on the "plug" of the $Zn-ZnSO_4-NaCl$ electrode. The proximal silver chloride pin may be seen inserted into the belly of the muscle through a "window" in the skin. The special clamp fixing the knee-joint to the floor of the moist chamber is also to be observed. Ordinarily the entire preparation is enclosed in a moist chamber. Adjustments of tension are made by moving the moist chamber up and down on the rigid racket-and-pinion stand seen in the figure.

the myograph and the optical system. Thus in the first "hole" of the myograph, 3.8 mm. from the axis, the magnification of the tendon movement on the plate was approximately 150. In a response in which 300 grams tension is developed (as in a twitch) the tendon movement occurring is approximately 0.022 mm. (12 mm. on plate = 1000 grams). In the 6th "hole" 17.7 mm. from the axis, where the magnification is only 30 the tendon movement would be approximately 0.52 mm. or more than 25 times greater.

The galvanometer leads as in previous experiments (1925a) were either two silver pins coated with chloride, or a combination of one silver chloride pin (proximal) with a $Zn-ZnSO_4-NaCl$ wick (distal). With this disposition of the electrodes the responses were usually approximately three-fourths monophasic.

I. Relation of the size of the action current to the duration of the mechanical response. When accurately recorded, the twitch of intact skeletal muscle has a characteristic flat "plateau" which terminates with great abruptness. This discontinuity has been referred to as the "angle" (Fulton 1925b, 1925d, 1925g; Fulton and Liddell, 1925b) and though its full significance has not yet been elucidated, it clearly provides a helpful and precise point for measuring the duration of the twitch. In what follows by "*duration of the twitch*" is meant the interval between the beginning of the electrical response and the "angle."

Since the terminal mechanical response of a short tetanus is also characterized by an "angle" (1925c), the interval between the beginning of the last action current of the tetanus and the tetanus "angle" corresponds with the interval used in measuring twitch duration. "After-action" has been used as a convenient term to designate this terminal interval in tetanic responses (Fulton 1924, 1925c) and by "*duration of after-action*," therefore, is meant the interval between the last action current of a tetanus and the beginning of relaxation (*i.e.*, the "angle").

It will be convenient first to consider the circumstances under which variations occur in the duration of the mechanical responses of skeletal muscle, considering at the same time the behaviour of the electrical responses. Since the stimuli are in all cases *maximal*, variations in the electrical responses signify alterations in size of the action current of each constituent muscle fibre. It is the purpose of this paper to consider the functional significance of these variations.

The alterations in size of the action currents which are about to be described are clearly physiological alterations and are not, for example, produced by variation (during the response) of distance between the galvanometer leads, for successive diminutions in size of electrical deflections during the ascent of a tetanus occur in gastrocnemius when there is absolutely no alteration of distance between the leads. Moreover, the maximum alteration of distance between the leads during responses

was, in the majority of experiments, less than 1 mm. which, when leads are separated by 2 cm., has a negligible effect upon the size of the recorded response. Nor can the alterations be attributed to the fibres becoming as they shorten more diagonally disposed with reference to the leads (Forbes, Ray and Hopkins, 1923) for corresponding alterations are found in sartorius, a parallel-fibred muscle, in which this could not occur (Fulton, 1925a). In view of Adrian's (1925) recent analysis of the action currents of the tenuissimus muscle, and his discovery that when the two leads are not taken from the same fibres in the muscle the resulting electrical response tends to be polyphasic in character, it may be urged that the action currents which I have recorded are merely the resultant potential of a heterogeneous collection of diagonal fibres, and consequently they do not represent faithfully the course of each individual electrofibregram. That the action current from the gastrocnemius is inevitably an algebraical resultant rather than a perfect summation is clear, but that the size and shape of the main deflection is not greatly modified by this circumstance is evident: since 1, parallel observations have, as already mentioned, been obtained from sartorius, and 2, since the alterations about to be described in the electrical responses were invariably accompanied by corresponding alterations in the *duration* of the mechanical response. The alterations in the electrical responses therefore are accompanied by physiological variations in the behaviour of the muscle.

1. Twitch duration and initial tension. The duration of the twitch increases, as is well known, when the initial tension (and length) of the muscle is increased within physiological limits (Fulton, 1925b). The size of the electrical responses likewise increases with the initial stretch, and passes through a maximum (as does also the tension developed) at very severe initial stretches. In my experience this is true of both the sartorius and gastrocnemius of the frog, and of the rectus femoris and gastrocnemius of the cat.

2. Duration of the second of two summated responses. A somewhat special case of increased duration of mechanical response is that occurring in response to a "supra-maximal" stimulus. Occasionally with a make induction shock or with a break when a core is in the coil (cf. Forbes and Gregg, 1915) two impulses separated (when they affect the muscle, see Forbes, Ray and Griffith, 1923) by an interval as small as 3.5 to 5σ may be set up by an apparently single stimulus.[2] Under these circumstances the height of the contraction is increased, as is well known, owing to summation, but the *duration* of the response is also increased and out of all proportion to the interval between the two stimuli. Two "small" electrical responses, resulting from a supra-maximal stimulus applied to a slack

[2] I have experienced great difficulty in eliciting in an intact frog a supra-maximal response with a break shock of a coreless coil, however strong the primary current was made. The sharper the break of contact (as then the speed of movement of the "contact" was increased by special levers) the more difficult it became, and in the majority of preparations supra-maximal responses could not be elicited at all by the break of a coreless coil. The fact that "supra-maximal" stimuli are readily elicited by "make" shocks may be put down either to hysteresis or (more likely) to chattering of the contact, as was pointed out by Forbes and Gregg (1915).

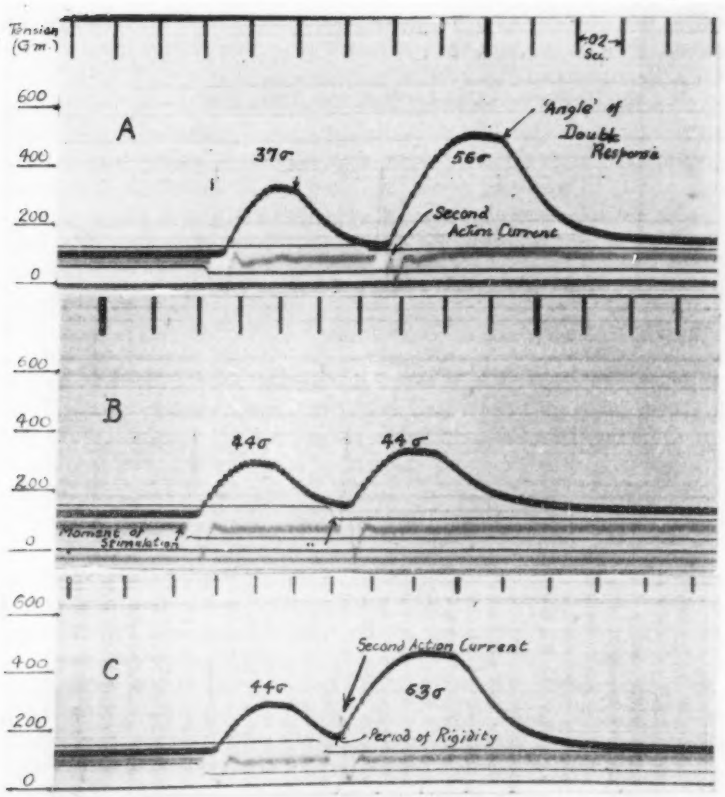

Fig. 2. Intact gastrocnemius of decerebrate frog showing electrical and mechanical responses to maximal and "supra-maximal" stimuli. Time above = 0.02 second. Heavy black line shows the myograph whose natural frequency (500 per sec.) can be seen. Single stimuli delivered to nerve at moment indicated by signal. The first was a "break," the second a "make." Line of zero tension at bottom of each figure.

A. T. = 24°. Duration of first mechanical response 37σ; the second response was double, the second action current of which commenced at approximately 4.5σ after the first, and the duration of the mechanical response is 56σ, *i.e.*, 50 per cent greater than the duration of the single response.

B. T. = 22°. Two *single* responses from another preparation to show that a second one, when single, is of approximately the same duration (in this case 44σ) as a first, (see previous paper on summation of responses).

C. T. = 22°. Same as B, taken immediately afterwards, in which the second response is double, the second action current following at 4.0σ after the first. Note the very slight string deflection produced by the second action current. The duration of the first mechanical response is 44σ the second 63σ.

muscle, are, therefore, in their relation to the *duration* of an ensuing mechanical response, roughly equivalent to on "large" electrical response such as occurs when the muscle is considerably stretched initially. In figure 2A the single response (at 24°) lasted 37σ and the double response 55σ though the interval between the two action currents on the double response was only 4.5σ. It thus requires much longer (other things being equal) for the excitatory ions liberated in the muscle by a double stimulus to diffuse away than the ions liberated by a single stimulus, even though stimulus II follows at a very brief interval. This assists in understanding the increased duration of mechanical response described in the previous paper (1925h), namely, the increase of duration of a second response when incident during the mechanical ascent or plateau of a first response. It was found that the duration of a second response was most augmented when it commenced about 5σ after the first, and that the power of augmentation progressively diminished as response II fell later and later along the course of response I. At the "angle" of response I the power of increasing the duration of a second response ceased completely—in other words, at the "angle" of response I a second response becomes of normal duration (1925h, p. 222). The diminishing degree of augmented duration of a second response as it occurs later and later in the course of a first has been interpreted as indicating the curve of concentration of excitatory ions (acid?) within the fibres as the first response progresses. If this interpretation be correct it follows that *the greater the concentration of activating ions within a muscle fibre at any given moment of time, the longer is the time interval required for their dissipation.*

3. Tetani and initial tension. Increase within limits of the initial passive tension (length) of a muscle *increases* in an ensuing short tetanus: *a*, the mechanical *tension* developed above the initial tension (Fulton, 1924; 1925a); *b*, the *size* of the successive electrical response accompanying the tetanus-plateau of the mechanical response ("plateau" electrical responses) (1925a); the *duration* of the successive mechanical responses as indicated by the duration of the terminal mechanical responses (after-action, 1925c). Here again the larger the electrical response the greater is the duration and size of the corresponding mechanical response.

4. Relation of twitch duration to the duration of the terminal mechanical response of a tetanus. The electrical response of a twitch of gastrocnemius muscle under appreciable initial tension is invariably larger than the electrical responses occurring during a tetanus plateau. One would, therefore, anticipate that the plateau mechanical responses would be correspondingly shorter in duration than the duration of a twitch, and such is in fact the case (1925c) for in a short tetanus the ratio of the twitch duration and the duration of the terminal mechanical response is approximately equal to the ratio of the amplitude of the twitch electrical

response and the terminal electrical response of the tetanus. That is:

$$\frac{\text{Duration of twitch}}{\text{Duration of after-action}} = \frac{\text{Twitch action-current}}{\text{Tetanus "plateau" action-current}}$$

These relations, however, hold only for short tetani (*i.e.*, 10 to 15 stimuli; see fig. 1 A of 1925c) when tetani are of longer duration especially when stimulated at a rate above that necessary to give complete fusion of responses, ions apparently accumulate within the fibres for the terminal mechanical response not, only loses its "angle" but may become in a tetanus of several seconds' duration two to three times longer in duration than a twitch.

5. Tendon shortening during tetanic responses. When at constant initial tension various degrees of shortening are permitted during short tetani, *e.g.*, by connecting the tendon to the horizontal arm of the myograph at various points from the axis, the contraction tension increases as the shortening permitted is made progressively less (1925a, 1925e). (See fig. 3.) Likewise the successive diminution in size of the individual electrical response of the series given by each compared tetanus becomes also less, so that the *electrical* responses during the tetanus plateau are largest when the tension is the greatest and the degree of freedom for shortening is the least. Thus, as shown in table 1 when but 0.05 mm. tendon shortening is permitted by connecting the muscle with the myograph at a point very near its axis (3.7 mm.) the tension developed in one experiment was 710 grams, and the ratio between the size of the "plateau" electrical responses and the first electrical response is 0.72 while with 1.03 mm. shortening (the muscle being attached 17.7 mm. from the axis of the myograph) the tension developed is 543 grams and the ratio between the first and the last action current 0.54. These responses are shown in figure 3.

This raises the question of whether successive diminution in size of the individual electrical responses during the ascent of a tetanus (1925a) can be prevented altogether. With intact sartorius, as in gastrocnemius, the more the shortening during the response the greater the successive diminution of the action-currents, but if sartorius is made to record a completely isometric response at a high initial tension no successive diminution of individual electrical responses is to be observed (1925e). In a completely isometric response of gastrocnemius, on the other hand, I have never found it possible to prevent successive diminution in size of the action currents at any initial tension—the highest ratio between a last and first electrical response of a tetanus observed under these conditions being 0.92. The failure to prevent diminution of action currents in gastrocnemius is almost certainly to be attributed to unpreventable shortening of the diagonally arranged fibres as contrasted with the paral-

lel fibres of sartorius, a point recently insisted upon by Fenn (1923) in accounting for the different thermal properties of the two muscles. In general, therefore, it may be said that successive diminution in size of the individual electrical responses during short tetani is at a minimum: 1, when tendon shortening is prevented; 2, when initial stretch is great; 3, when the fibres of the recording muscle are parallel. This can leave but one conclusion, namely, *that successive diminution of tetanic action currents is due to shortening of the individual fibres.* The reverse of this, namely, *lengthening* of the fibres during a response should cause an increase in size of the electrical responses. This has been investigated in-

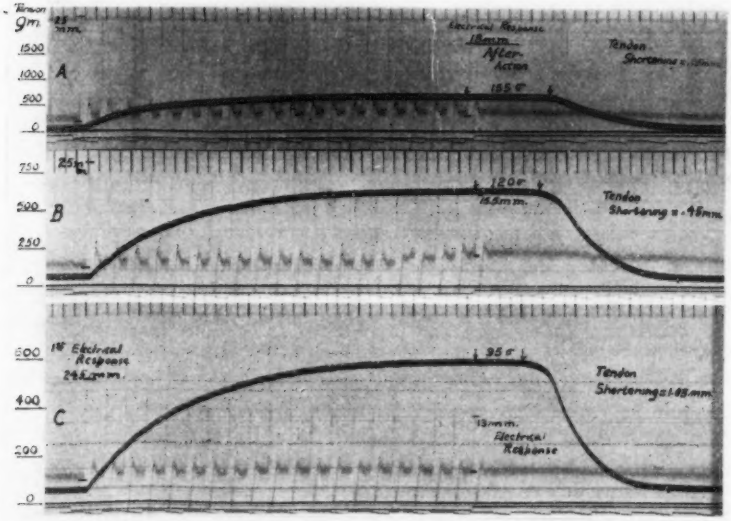

Fig. 3. Three tetanic responses of an intact gastrocnemius of 25 gram "winter" frog in which the amount of tendon shortening occurring during the response has been varied. In A the tendon shortening (0.05 mm.) was the least, etc. The responses of this record have been analysed in the lower rows of table 1. It will be seen that where the least shortening occurs during the response there is the least diminution in size of the successive action currents and also the longest duration of terminal mechanical response (indicated on the figure by arrows). The size of the first electrical response of the tetanus is nearly the same in all three cases, which is a control to the constancy of the initial conditions (length, etc.) in the three records. Time above = 0.02 second. String tension 8 mm. per mv. in all three records. Below line of zero tension is the short-circuiting signal controlling stimulating electrodes.

In A, 1 mm. vertical distance = 91 grams tension.
In B, 1 mm. vertical distance = 28 grams tension.
In C, 1 mm. vertical distance = 18 grams tension.

cidentally in experiments on root stimulation of gastrocnemius (Fulton, 1925f). If one lumbar root, "B," is stimulated for a brief interval concurrently during the plateau caused by tetanization of the other root, "A," the muscle, as a result of the new fibres brought into play by the second root, shortens further (fig. 3, 1925f). During the after-action from the stimulation of B, the action currents of fibres controlled by root A are smaller than before the stimulation of B owing to the more shortened state of the fibres. But during the relaxation from the response of B, *i.e.*, when the (still active) fibres of A (and B) are lengthening, the electrical responses *increase* progressively with the lengthening of the muscle. It will be seen that these observations are in keeping with those of Samojloff (1908) in which he found that in two fused responses (excised muscle)

TABLE 1

Showing the effect of moderate tendon shortening at constant initial tension (50 grams) upon the electrical responses, the tension developed during tetanus and upon the duration of the terminal mechanical response (after-action) of short tetani. The responses from which the lower group of measurements were made are shown in figure 3. (Gastrocnemius muscle; 25 gram frog; T = 14°); string tension, 50 m. per amp. in first; 55 in the second)

TENDON SHORTENING	TENSION	ELECTRICAL RESPONSES			DURATION OF AFTER-ACTION
		First response	Last plateau response	Ratio	
mm.	*gm.*	*mm.*	*mm.*		
0.06	840	17.7	13.0	0.73	140σ
0.55	800	17.8	10.5	0.60	100σ
1.30	760	17.8	9.2	0.52	90σ
0.05	710	25	18	0.72	155σ
0.45	595	25	15.5	0.62	120σ
1.03	543	24.5	13.0	0.54	95σ

no diminution occurred in the size of the second action current if the response were made rigidly isometric.

The alteration in size of the electrical responses in the experiments in which varying degrees of tendon shortening were permitted leads one to inquire whether there is a corresponding alteration in the duration of the *final* mechanical response, *i.e.*, in the *after-action*.

The influence on the final mechanical response is unmistakable as regularly observed in twelve different preparations. Thus, as shown in table 1, and in figure 3 the after-action is 155σ when but 0.05 mm. shortening had occurred, and 95σ with 1.03 mm. shortening. In another preparation with 0.06 mm. shortening the after-action was 140σ and with 1.03 mm. 90σ (ratio 1.5). In these responses, as shown in table 1, the size of the corresponding *terminal* electrical responses were 13.0 mm. and 9.2

mm. (ratio 1.4). The ratios of the electrical responses in the two cases compared with the ratios of durations of the mechanical response show a close similarity and thus bear out an observation (1925c, p. 441) *that the duration of the mechanical response varies closely with the size of the electrical response.*

The mechanical responses shown on figure 3 possess another feature of interest: namely that the "angle" is much sharper in the response in which the least shortening has occurred. An explanation of this suggests itself from the fact that many of the fibres in gastrocnemius are diagonally disposed. Those which are inserted most diagonally into the tendon and the tendon aponeurosis will for simple mechanical reasons be more affected when the tendon is allowed to shorten than those which run parallel with the tendon. The result will be that the cessation of contraction will not be synchronous and the "angle" of the responses of the individual fibre will be smothered in the summated and therefore rounded "angle" of the whole response. This explanation is supported by a fact often observed in gastrocnemius that the "angle" of a second response which summates with a first is invariably less sharp than the "angle "of the first response alone (see fig. 7 of another paper, 1925g). In a *parallel* fibred muscle, on the other hand, such as the rectus femoris (cat) modifications in the "angle" with increasing degrees of shortening have not been observed.

Significance of diagonally arranged fibres. A corollary of these considerations is that a diagonal fibred muscle is most effective in developing tension when no shortening occurs. In fact if extensive shortening takes place the fibres tend to pull against themselves rather than on the tendon. Diagonal fibred muscles, especially when their arrangement is pennate, may therefore be regarded as primarily "tension" muscles in contrast with the parallel-fibred "shortening" muscle of which nearly all those inserted or having origin in the hyoid bone of mammals are excellent examples. These facts were largely realized and admirably set forth in 1667 by Nicolas Steno[3] the celebrated Danish physician and physiologist, and they were later extended but somewhat modified by Borelli (1680).

Bearing upon the question of diagonal arrangement of fibres in muscles

[3] Though the admirable later work of Borelli (1680) on the mechanics of muscular activity is remembered and often referred to, that of Steno seems to have been somewhat neglected. In a little quarto volume entitled *Elementorum Myologiæ Specimen* first published in Florence in 1667 and subsequently in Amsterdam in 1669 and 1685, accurate descriptions are to be found of the arrangement of the muscle fibres in a large number of muscles in the human body together with diagrammatic parallelograms of forces in the cases of those in which the fibres are diagonally disposed. A copy of the Florence edition, 1667, is in the possession of Prof. T. Graham Brown of Cardiff.

is the interesting observation (Marey, 1873) that the fibres in the gastrocnemius of a negro are more nearly parallel than the fibres in a white man's gastrocnemius. The negro's gastrocnemius is therefore more a "shortening" muscle than a white man's which is more a "tension" muscle.[4]

6. Duration of submaximal responses. In submaximal tetanic responses as when only one nerve root of the double (or triple) root supply of gastrocnemius is stimulated (Fulton, 1925f) the duration of the terminal me-

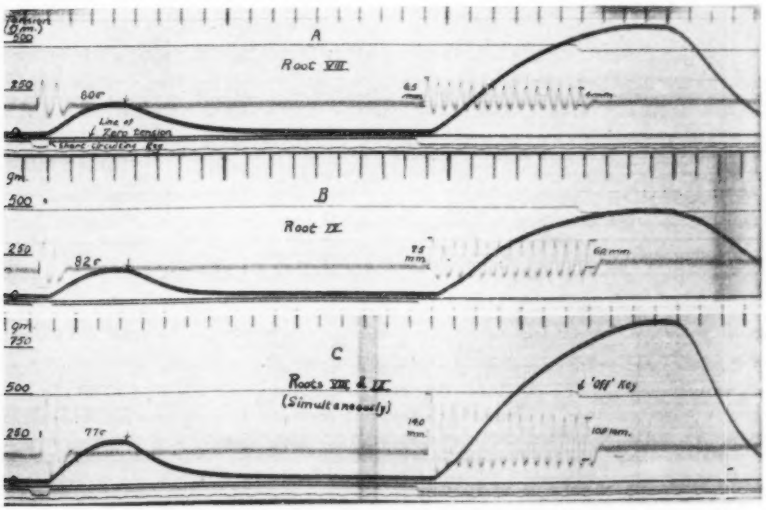

Fig. 4. Responses of intact gastrocnemius to stimulation (100 per sec.) through roots VIII and IX separately (A and B) and then simultaneously (C). The curious somewhat prolonged relaxation following stimulation of root IX is noteworthy. The summation of electrical effect when both roots are stimulated simultaneously may also be noted. First responses double. The lower vibrating signal (50 per sec.) shows *half* the rate stimulation (100 per sec.) applied to the nerve. Other signals are the "on" and "off" short-circuiting keys. Time above = 0.02 second. String tension 7 mm. per mv.

chanical response tends to be slightly longer than when the muscle is stimulated (maximally) simultaneously through all its roots. In keeping with this the summed magnitude of any two corresponding electrical deflections evoked when the single roots are stimulated one after another is usually somewhat greater than the corresponding electrical responses of a maximal tetanus, the overlapping being 3 to 10 per cent (1925f).

[4] I am indebted to Professor Sherrington for directing attention to Marey's observation.

Thus in the double responses reproduced in figure 4 the duration when root VIII was stimulated was 80σ, that of root IX 82σ and that of the maximal response 77σ. The wholly different shapes of the curves of relaxation of the two submaximal tetani is worthy of note. The slower relaxation in the middle record suggest that the majority of the fibres involved in the reaction were parallel and hence shortened but little. This characteristic difference in the rates of relaxation from the responses of the separate roots has been observed in the majority (but not in all) experiments on the point (fifteen).

It is noteworthy in figure 4 that of the separate responses of the two roots the greater tension is developed in response to root XIII which, however, has the smaller electrical responses of the two. This again favours the inference that in this case the fibres innervated by root VIII were more diagonally disposed than those receiving nerves from root IX and therefore better adapted for tension development. In rather more than half the experiments on root stimulation the root which caused the greater tension development produced the smaller action currents.

Discussion. In the six circumstances just described in which alteration of the duration of the mechanical response has been observed, parallel alterations have in each case been found in the size of the action current, and this has led to the inference that an intimate causal relation exists between the size of the action current and the duration of the mechanical response. A large electrical response presumably indicates a large initial breakdown of energy (Fulton, 1925a; 1925e; see below, section III) and a longer time is apparently required for a large number of the "contractile" ions so formed to dissipate themselves than for a smaller number.

The conditions (except 2) in which parallel variations in the electrical and mechanical durations have been observed have one common feature—namely, varying length of the muscle fibres:—thus in the twitch at various initial tensions, in motor tetani, in twitch and after-action, in the tetanic responses with varying degrees of tendon shortening, and in the submaximal responses the *longer the fibres* (*i.e.*, spatial length) *during the responses the greater is the duration of the mechanical response and the larger the action current.* The alterations in length of the muscle as a whole may be as small as 0.1 mm., *i.e.*, less than 0.5 per cent of the total length, and still produce a measurable difference in the duration of the response.

This leads one to inquire whether under any circumstances the duration of the mechanical response varies independently of the action current.

II. The influence of shortening on the duration of the twitch. The only well-substantiated instance in which the duration of the mechanical response has been found to vary independently of the electrical response is in the case of the twitch in which varying degrees of tendon

shortening are permitted, during the (single) response. The conditions and results of these experiments, therefore, deserve careful consideration and analysis.

The effect of varying the degree of tendon shortening occurring during a twitch has been investigated by recording responses in which the muscle was attached to the myograph, as just described, at various distances from the axis,—the initial tension of the muscle remaining constant. In ten experiments in which the tendon shortening occurring during the twitch has been varied it was found that *the smaller the shortening permitted*

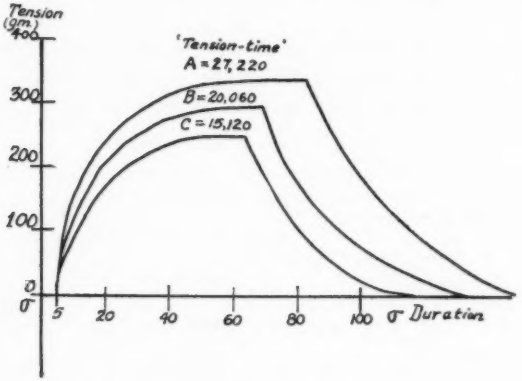

Fig. 5. Twitches of an intact frogs gastrocnemius at 15° in which varying degrees of tendon shortening occurred during the responses, which have been plotted on the same tension-time coördinates. The product of the tension × the time (*i.e.*, the area) is indicated above each curve. The initial tension was constant (50 gm.) in each response.

	Tendon shortening *mm.*	Tension developed *gm.*	Duration to "angle" *0.001 second*
A	0.025	332	82
B	0.21	295	68
C	0.41	244	63

Stimulus a maximal break shock applied to sciatic nerve.

the greater the tension developed and the longer the duration of the response measured to the "angle." In figure 5 three responses of the same preparation taken at short intervals and at the same initial tension are plotted on the same tension-time coördinates. It will be seen that as between a twitch with 0.02 mm. tendon shortening and one in which 0.52 mm. occurred there is a difference of more than 25 per cent in the tension developed and a similar difference in the duration of the respective twitches. This in other words amounts to a diminution of nearly 50 per cent in the total area ("tension-time") of the response. In some experiments even

greater differences have been noted: in others it has been somewhat less (see table 2).

It may be urged that tension-time is a purely abstract conception having no relation to the power of a muscle to do work; but, as Hartree and Hill (1921a) have emphasized, this is certainly untrue, for the "tension-time" of a contraction is a direct measure of the power of a muscle to give momentum to a heavy mass. The tension-time may therefore be taken as an index of the capacity for doing work.

In these responses the initial tension and length of the muscle was the same in all three cases, and the action current was therefore also of constant size.

One may naturally enquire as to the relation between the enhanced duration of the twitch when the shortening is small and the corresponding enhanced duration of the final mechanical response of a tetanus (after-

TABLE 2

Showing the effect of varying degrees of tendon shortening occurring during the twitch of gastrocnemius (frog)

EXPERIMENT	APPROXIMATE TENDON SHORTENING					
	0.02 mm.		0.10 mm.		0.52 mm.	
	Tension developed	Duration	Tension developed	Duration	Tension developed	Duration
	grams	*0.001 second*	*grams*	*0.001 second*	*grams*	*0.001 second*
10, iv, 25 (18°)	332	82	295	68	244	63
25, iii, 25 (11°)	275	160	260	125	245	117
11, iv, 25 (16°)	360	80	270	69	222	61
28, iii, 25 (15°)	400	90	310	71	250	64

action) described above. The latter has been associated with increased size of the electrical responses during the tetanus plateau which in turn depends upon greater length of fibre, but *not* upon shortening during the response since no shortening occurs during the tetanus plateau of which the after-action is a part. With the twitch, however, shortening *during the response* is the only variable as in all three cases the muscle is *initially* at the same length and tension. When two summated responses are recorded as in figure 6 both factors (altered electrical response and varied shortening during the response) appear to be involved in determining the duration of the second response. Thus the durations of response II in the three cases were: 82, 72 and 63σ. Other details are given in the legend.

Submaximal responses: The behaviour of the twitch when varying degrees of shortening occur throws light upon a phenomenon frequently observed in the course of these investigations, namely, that *submaximal*

responses measured to their "angle" *are usually of slightly greater duration than maximal responses.* Thus it was found that when either single root of the supply to gastrocnemius was stimulated the duration of the response was regularly somewhat greater than that of the response evoked

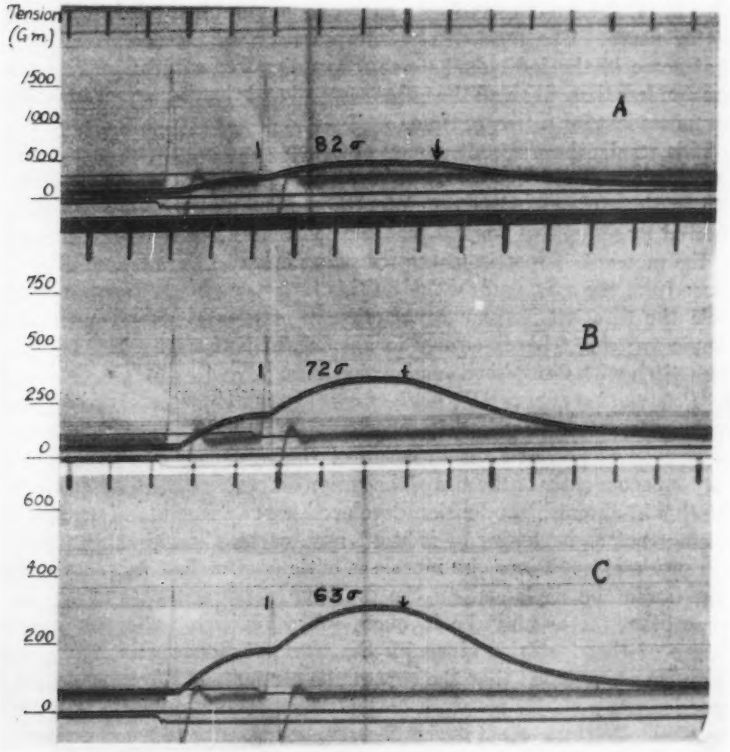

Fig. 6. Double response of frogs gastrocnemius in which various degrees of tendon shortening occurred. The first electrical response is approximately constant in the three responses while the second one is smallest in C in which the most shortening has occurred. The durations of the second response in A, B and C are respectively 82, 72 and 63σ. The stimuli were both break shocks given by magnetic signals whose shadows show on the plate above and below the galvanometer string. Time above = 0.02 second. String tension 8 mm. per mv.

when all roots were stimulated simultaneously. In figure 1 of a previous paper (Fulton, 1925f) the durations of the twitch as provoked by roots VIII and IX were respectively 58σ and 59σ; while both roots together gave a response but 56σ in duration. Compare also the duration of the

double "twitches" in figure 6. Similarly in responses of mammalian rectus femoris (obtained by Liddell and the author, 1925b) on stimulation of the anterior crural nerve by submaximal shocks, the durations of the twitches were respectively 40.5, 40.0, 36.5, 35.5, when the following respective tensions were developed: 800, 800, 1000 and 1000 grams. This has also been observed with submaximal stimuli applied to the frog's motor nerve. The most likely interpretation of these observations is that some of the individual fibres responding to a submaximal stimulus shorten less than when all the fibres respond and therefore their individual responses last for a greater time.

This recalls the old controversy of Schenk and Blix (see Lucas, 1904; and Biedermann, 1896, vol. i, p. 78), as to whether an arrested isotonic contraction relaxed sooner or later than a normal contraction. Lucas showed by an optical method that the controversy turned upon the ratio of the moment of inertia of the recording lever to the mass at unit distance from the axis, and that when this ratio were made as small as possible the arrested isotonic contraction is of greater duration than the simple twitch. This is clearly in keeping with the greater duration of the twitch with diminished tendon shortening.

"Potential" energy and the Fenn effect: The twitch, when executed under various degrees of freedom for shortening, provides a case in which the tension developed and the duration of the mechanical response clearly vary independently of the size of the action current. That tendon shortening should diminish the tension developed is not surprising, but it is surprising that a shortening of only 0.5 mm. causes the tension to diminish so considerably. Since the muscle is initially stretched this loss of tension cannot be explained on the grounds of having to take up slack. Some other factor must be invoked. A suggestion as to the possible nature of this factor is given by the work of Gasser and Hill (1924). They have concluded that the contractile elements of a muscle are to be regarded as an elastic net-work bathed in a viscous medium. It is clear, therefore, that the greater the shortening occurring during a response the more will be the energy dissipated internally as heat in overcoming the viscosity of the muscle substance. In a muscle with diagonally arranged fibres the myriad of frictional and viscous surfaces which would be affected when very minute alterations in shape occurred,—as when the tendon shortened 0.5 mm.,—would certainly be sufficient to produce a large percentage diminution of the external tension developed in the tendon. Whether these impeding forces would be sufficient to account for the observed loss of 25 per cent in the total tension (fig. 5) developed cannot be determined (since the surface area and coefficient of friction is not known) but it does not appear inherently improbable. According to this view a quantum of potential energy is liberated by a

given stimulus, the absolute size of the quantum being determined by the initial length of the muscle and documented by the size of the action current. The *internal* or "potential" tension energy capable of being developed by the contractile elements at a given length is presumably the same in all cases, but when extensive *internal* resistance is met with, as when shortening occurs, the *external* tension developed diminishes. Now it is essential to understand exactly what is meant by the internal or "potential" energy. When a muscle contracts under completely isometric conditions the irreversible degradation of potential energy is relatively smaller than when shortening occurs. This suggests that the fate of the potential energy varies with the mechanical conditions obtaining during the contraction, and many recent investigations point to the fact that part of the potential energy of an active muscle may become reabsorbed as free energy. To put it less accurately but in more tangible language, a quantum of lactic acid is liberated by a stimulus; if the response is isometric part of it reverts to the precursor without appearing as heat; when shortening occurs more of the total lactic acid is utilized. As Hill (1925) has pointed out: "The common view is that, in relaxation, the whole of this mechanical potential energy, if not previously employed in doing work, is dissipated into heat. Such a view, however, is not necessary, and one might well be led to look for a way in which the organism could avoid such an obviously uneconomical procedure. It would seem possible that part, at any rate, of the free energy of the muscle, while in a state of active tension, is used in commencing to carry out the recovery process which has later to be completed by oxidation" (p. 261). These deductions are based upon Fenn's (1923) recent discovery of excess energy liberation by muscles when they shorten. He finds that when a muscle shortens, lifting a weight, the *total* energy liberated as heat is approximately the same as the heat liberated in an isometric contraction; therefore in shortening the muscle must have liberated an extra amount of energy equivalent to the work done in lifting the weight. This result has been amply confirmed by Hartree (1925). Thus *more energy (heat plus work) is liberated when a muscle shortens than when it contracts isometrically.* Yet, as judged by the tension-time of the response, the external energy appearing at the tendon is *less* when the muscle shortens than when it does not. It is evident therefore that when shortening occurs contraction is carried out with less efficiency,—at least if the tension-time can be used in a measuring efficiency.

Perhaps the most convincing evidence that the fate of the potential energy liberated in a muscle varies with the mechanical conditions encountered after the stimulus is over, *i.e.*, during the response, is the fact that when the muscle shortens the *duration* of the response diminishes. The potential energy (lactic acid) is in other words dissipated

most slowly when there is no change in shape of the fibre. There is reason to believe that the lactic acid is liberated more or less explosively (Hill, 1925), and since it is produced by a reversible reaction, there will be a constant tendency for it to be reformed into glycogen (or some other "precursor"). When, as in an isometric contraction, the muscle cannot change its shape, the activating ions are not so quickly "neutralized," —owing presumably to less ample opportunity of their coming into contact with neighbouring interfaces,—and more time is allowed for the ions to be reconverted into the precursor. When, on the other hand, shortening occurs, the response being more brief, fewer of the ions are reconverted and more of the potential energy becomes utilized. It would seem that only along some such lines as these can Hill's results and the observations recorded in the present paper be harmonized with the "Fenn effect." It may be added that the recent criticism of the "Fenn effect" offered

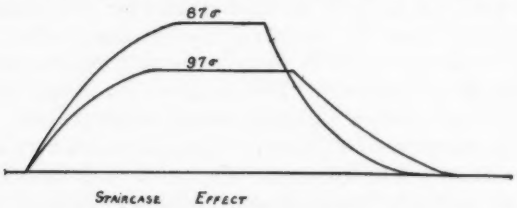

Fig. 7. Two successive twitches plotted on the same coördinates. The lower one, whose duration was 97σ was taken under exactly the same mechanical conditions as the upper one but was the first response after a prolonged rest. The area of this one is 808 (97 $\times$ 7.3). The upper one, whose duration was 87σ was taken two minutes later, the muscle having been stimulated in the interval by a brief tetanus. The area of the upper twitch is 870 (87 $\times$ 10).

by Tiegs (1924) does not appear justified, since "viscous heat," though its magnitude was probably underestimated by Fenn, is clearly the result of chemical breakdown. If resistance is overcome, whether the resistance be internal or external in origin, it is accomplished through the contractile activity of the lactic acid mechanism. When the internal resistance encountered is large the tension appearing at the tendon is correspondingly reduced. Consequently it is not legitimate to assume that viscous heat arises *de novo* without a chemical background of energy liberation.

The staircase effect: The distinction between external and internal tension which has just been made may possibly throw light upon the phenomenon of the "staircase." It will be recalled (Fulton, 1925b) that a single response of an intact frog's gastrocnemius occurring after a prolonged rest is of much greater duration than usual, and, as is well known, it develops *less* tension than in succeeding responses. Thus in figure 7 two responses of the gastrocnemius of a 40 gram frog are plotted just

before and just after a short tetanus. The duration of the first is 97σ, that of the second 87σ, but the tension of the latter is 25 per cent greater than the former. *This is precisely what would happen if during the response resistance (internal) were encountered to the development of tension.* The individual response would become longer and less (external) tension would appear. This suggests that after a prolonged rest the muscle has, as it were, become "set," *i.e.*, the resistances encountered when first thrown into activity are greater for the first response than for succeeding responses. Some impedence of this sort would seem in the nature of things almost inevitable. It will be recalled that there is no staircase effect in the electrical responses—in fact the reverse is usually the case—the electrical responses become as a rule somewhat smaller with successive"twitches" in which the staircase shows itself (see fig. 6 of previous paper). This interpretation of the staircase places the phenomenon entirely upon a mechanical basis. If correct it is clear that when the contractile elements of a muscle encounter resistance to shortening whether internal or external in origin then response is more prolonged. As pointed out elsewhere (1925b) this view of the staircase phenomenon makes improbable the existence of a supernormal phase of contractility.

The all-or-nothing theory: Though many recent authors (Fenn, 1923, p. 199; Hartree and Hill, 1921b; Judin, 1923; Athenasiu, 1922) have objected to the all-or-nothing law as applied to muscle fibres on the grounds that energy liberation in muscle is not constant, attention may be expressly called to the fact none of the observations just recorded cast any reflection upon it. The theory as originally put forward by Pratt (1917), Pratt and Eisenberger (1919), Lucas (1909), and others infers in the case of muscle that when a stimulus is adequate, a muscle fibre contracts maximally or not at all. And under given mechanical conditions the amount of the contraction does not increase with increasing strengths of stimulus (Forbes and Cattell, 1924, p. 166). The all-or-nothing law as originally put forward explicitly made no inferences as to what might occur when the mechanical conditions of the muscle were altered. Given constant mechanical conditions the size of the electrical response and the size of the mechanical response appear to vary directly with the number of units stimulated (Watts, 1924). With these considerations in mind it is impossible on the grounds of any existing evidence to impeach the all-or-nothing theory as applied to muscle (Adrian, 1922). Nevertheless, as Fenn (1923) has pointed out, the limitations of the theory must always be borne in mind in order to prevent misunderstanding.

III. THE CORRELATION OF THE SIZE OF THE ACTION CURRENT WITH ENERGY LIBERATION AND INITIAL HEAT PRODUCTION. The functional significance of the action current in muscle appears to have received inadequate attention, and the prevailing view concerning it (which has

arisen largely as a result of the all-or-nothing theory) would seem to be that expressed by Forbes, Ray and Hopkins (1923 p. 301) that: "the action current in response to a single stimulus, may have little or nothing to do with the tension actively developed by the contraction of the muscle, for it is well known that this action current has passed its maximum before the mechanical response has appreciably altered the tension." It is now clear on many grounds that this, the prevailing view, does not represent the whole truth and that a more fundamental significance is to be attached to the action current.

Latency: The interval between the beginning of the action current in muscle and the commencement of the mechanical response is so brief that several recent authors who have employed technique of enhanced accuracy (Einthoven, 1924; de Jongh, 1923; Roos; Lewis, 1925, p. 377–378) have been inclined to regard the beginnings of two responses as practically simultaneous. A torsion-wire myograph of 1600 per second natural vibration frequency, has shown that the interval between the beginnings of the electrical and mechanical responses of *intact* frog's muscle at 20° may be as low as 1.5σ, but never in my experience are the two actually simultaneous.[5] It happens that this interval of 1.5σ is but little shorter than the duration of the rising phase of the action current at 20°, and it has therefore been suggested that the first mechanical alteration in the muscle does not occur until the wave of permeability (of which the action current is an index; Hartree and Hill, 1921, p. 140) has nearly reached its height. For our present argument it matters very little whether the duration of the true latency and the rising phase of the action current happen to coincide or not; the *important point is that the beginning of the electrical response does invariably slightly precede the mechanical response.* Now the ionic interchange brought about by this relatively brief period of increased permeability presumably leads to the break-down of acid substances from some "precursor." Chloride ions, for instance, are known to flow into muscle fibres when in activity (Embden and Lange, 1923) and other ions (*e.g.*, phosphate) probably flow out, thus shifting the hexose-phosphate-lactic acid equilibrium in favour of lactic acid formation.

We have seen that the size of the action current as well as the duration of the mechanical response vary closely with the length of the muscle fibre, being greatest when the muscle has reached its physiologically maximum extension. Increased size of action current in individual fibres, therefore, gives evidence of an increased ionic interchange. Whether this is due to an enhanced duration of the wave of permeability or to increased potential difference between the two sides of the membrane

[5] In mammalian muscle this latent interval may be as brief as 0.6σ.

has not yet been settled (Fulton, 1925a) but it suggests that the size of the action current indicates the extent of the initial process of breakdown, especially since the duration of the mechanical response increases with the magnitude of the electrical deflection.

Heat: A. V. Hill (1925) has recently investigated the effect of length during tetanic responses upon the (initial) heat produced and the tension developed in the isolated sartorius muscle. He allowed the muscle to contract isotonically from a length "100" to "95," "90," etc., down to "50" and then caused it to record an isometric response. He found that heat and tension tended to diminish *pari passu* with the length until the length had become about 50 per cent of its resting value,—the approximate length assumed by a completely unloaded muscle. The heat and tension curves, however, usually showed slightly different maxima. If now one examines the behaviour of the electrical responses it is at once apparent that under corresponding conditions they diminish qualitatively and, as far as one can determine, also quantitatively, as the initial heat production. Let us take for an example the alterations when varying degrees of tendon shortening are permitted. In sartorius when no shortening is permitted there is no successive diminution in the electrical responses. If a small amount of tendon shortening occurs (*e.g.*, 0.1 mm.) the electrical responses diminish during the shortening and become of constant size *at the new length*. The action currents are thus smaller at the shorter length.

A closer parallel to Hill's experiments, however, are those in which various degrees of tendon shortening were permitted before becoming "taut" (Fulton, 1925a). In these experiments the size of the electrical responses accompanying the mechanical plateau has always varied closely with the tension developed which in turn decreased with the length. This is shown in figure 7 and table 3 of a previous paper (1925a). The extent of the diminution of the plateau electrical responses with diminishing length has in some experiments been as great as 50 to 60 per cent, or approximately the degree of diminution which Hill found in the heat production.

Another parallel between heat and electrical change is found in experiments in which isotonic records were taken in which the after-loaded muscle[6] lifted increasing weights, not to a constant height but as high as it could lift them. In these the successive action current become augmented (fig. 8 of 1925a) during the period in which the muscle is developing tension, and the degree of augmentation increased with the weight.

[6] The weight rested on the after-loading screw in such a way that the muscle was initially under only 1 to 2 grams but on shortening picked up the weight.

From these comparisons with heat production especially with those recently performed by Hill in which the conditions of the experiments were closely parallel to those under which the electrical responses of the present investigation have been recorded, the conclusion would appear unavoidable that the initial breakdown process in muscle as measured by heat production runs parallel with the size of the electrical responses, *and that the physico-chemical change which produces the action current likewise determines the amount of "potential" energy liberated in the initial breakdown process.* It may be repeated that potential energy, as Hill (1925) has emphasised, is not necessarily synonymous with the total energy liberated as heat or work, for a portion of the potential energy appears to be reabsorbed as free energy (especially in isometric responses) during the kinetic phases of the response. The conclusion therefore is not in any way out of harmony with the conclusions of Fenn (1923).

It is evident that if heat and electrical energy run parallel the electrical method of studying the initial energy changes in muscle yields certain information which cannot readily be obtained by the thermodynamic procedure. In the first place, the electrical method may be utilized in the intact animal since with this method there is no need to maintain the temperature absolutely constant. Secondly, one may study successive modifications in the potential energy liberated by each stimulus—as in a tetanus in which shortening occurs, and this is impossible in heat analysis. Thirdly, one may record simultaneously by the electrical method the mechanical response and index of energy potential liberation, while with heat determination there is an inevitable lag which renders the exact chronological relations difficult to ascertain. Finally, in heat records the contractile energy dissipated as heat in overcoming the internal viscosity of the muscle in shortening, cannot easily be distinguished from the heat of the initial breakdown process—a fact which renders experiments on heat production when shortening occurs difficult of interpretation—whereas in the electrical method, one has less complicated index of the potential energy liberation during (or preceding) the initial breakdown processes.

Other parallels between heat production and the size of the action current might be brought forward, but it will be more profitable to deal with them in answering possible objections which may be raised against the view that the electrical change is an index of the initial breakdown process.

The conclusions of McSwiney and Mucklow (1922) as to the lack of parallelism between electrical change and heat do not affect the arguments given above since they did not attempt to analyse the successive increments of electrical variation and since also they made no allowance in comparing curves obtained at different temperatures for the increased *duration* of the electrical response at low temperatures. It is impossible

to measure or to compare summed potential differences by a ballistic method when the duration of the potential difference varies. They have not yet examined the effect of fibre length on the summed potential difference, but Doctor McSwiney has very kindly informed me that he is preparing to investigate this question in the near future.

Hill (1913) found that increasing of the stimulation rate above 30 to 40 per second does not materially increase the heat liberation of isolated sartorius and it may be urged, in as much as the *intact* frog's gastrocnemius responds electrically up to 120 or more per second and becomes fatigued more quickly at this high rate than at a low (Fulton, 1925c) that the electrical changes in this case do not run parallel with heat. In reply to such an objection it may be said: 1, that the individual electrical variations are smaller by about 10 to 15 per cent when the muscle responds at 100 per second than when responding at 50; 2, Hill's experiments have been performed on excised muscle in Ringer solution and were stimulated directly. In unpublished experiments on the effect of temperature I have repeatedly observed that when a muscle (even when intact with circulation) is immersed in accurately compounded Ringer solution the mechanical responses become prolonged in duration and greatly modified in shape (less abrupt ascent, rounded crest and smothered "angle") and the minimum summation interval is usually increased. Moreover when the excised sartorius of a frog is placed in Ringer and stimulated at 100 per second it not infrequently responds at half the rate of stimulation, *i.e.*, 50 per second or if the strength of stimulation is greatly increased and in this way caused to respond at the full rate the electrical responses are of considerably reduced magnitude. These observations make it evident that a muscle in Ringer solution cannot be regarded—electrically, at least—as a normal tissue and it is therefore unjustifiable to compare its responses with those of an intact preparation. Moreover, without a record of the electrical responses one does not know whether a muscle actually responds at the rate at which it is being stimulated. If it can be shown that a muscle which is stimulated at 80 per second *responds at 80 per second with full-sized electrical deflections*, but with no greater heat liberation than at 40 per second, then this objection may prove of consequence.

SUMMARY

Simultaneous electrical and mechanical records of enhanced accuracy have been obtained of the responses of *intact* skeletal muscle (active circulation) to indirect *maximal* stimuli,—single and tetanic. A study has been made of the influence of initial fibre length and of shortening during activity upon the mechanical and electrical responses. The following are the chief conclusions:

1. The size of the electrical response and *duration* of the mechanical response increase (without certain limits) with the length of the fibre. Six conditions are described in which the size of the electrical response and the duration of the mechanical response were found to run a parallel course.

2. The smaller the tendon shortening permitted during short tetani: *a*, the larger the electrical responses accompanying the mechanical plateau of the tetanus; *b*, the greater is the tetanic tension; *c*, the longer the duration of the terminal mechanical response; *d*, the sharper (in a diagonal fibred muscle) is the terminal "angle" of the response.

3. At constant initial length (and therefore constant size of action current) the smaller the tendon shortening occurring during a *twitch: a*,the greater the tension developed; *b*, the *greater the duration* of the response measured to the "angle." The activating ions are therefore dissipated most slowly when no shortening occurs.

4. Submaximal responses are of greater duration than maximal responses.

5. In a diagonal fibred muscle the greater the tendon shortening taking place during a response (e.g., a twitch) the more unequal will be the extent of shortening occurring among the individual muscle fibres. Since the duration of a twitch diminishes *pari passu* with increasing shortening—the cessation of contraction among the individual muscle elements of a diagonal fibred muscle will tend to be more and more asynchronous as the tendon shortening during the response becomes greater and greater. This accounts for the increased smothering of the "angle" of the mechanical response of gastrocnemius as shortening increases.

6. The muscles with diagonally arranged fibres are primarily "tension" muscles; those with parallel fibres are primarily "shortening" muscles (Nicolas Steno, 1667).

7. The size of the action current and initial heat production (as investigated by A. V. Hill) diminish *pari passu* with the length of fibre. It is inferred from this and from other evidence that the electrical response provides an uncomplicated index of the initial breakdown process in muscles (potential energy liberation).

8. Reasons are given for believing that the initial "potential" energy liberation in a given response is determined by the length of fibre *at the moment of stimulation*. The diminished tension observed in a twitch when shortening occurs, according to this interpretation, is due to the dissipation of contractile energy as heat in overcoming the internal viscous and frictional resistances of the muscle necessary to shortening.

9. The observations cast no reflection upon the all-or-nothing theory as applied to muscle fibres.

10. The staircase effect is discussed in the light of these experiments,

and reasons are given for regarding it as a purely mechanical effect, due to diminution with successive stimulation of *internal* frictional resistances.

11. In isometric contraction a portion of the potential energy liberated appears to be reabsorbed as free energy (of the precursor), while in isotonic contraction more of the potential energy is utilized in the course of the response. The mechanism of the "Fenn effect" is discussed in the light of these inferences.

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THE INSEPARABILITY OF MECHANICAL AND ELECTRICAL RESPONSES IN SKELETAL MUSCLE

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The possibility of separation of the electrical responses from the contractile process has long been a moot question. Mines (1913a; 1913b; 1914) as is well known, insisted that the mechanical response could be separated from the electrical response by means of electrolytes. He found, for instance, that after the beat of a frog's heart (auricle and ventricle) had ceased following perfusion with a Ca-free perfusate, rhythmic electrical response still remained. And more recently Beritoff (1924a; 1924b) has reported that after severe fatigue the electrical response may still remain when the mechanical response has disappeared. Recent work, however, has tended to discountenance Mines' belief that the contractile and the excitatory processes are independent and separable. De Boer (1917) found in water-rigor both of skeletal and heart muscle that owing to swelling of the fibres contraction could not be recorded on account of edematous shortening already present. He suggests in view of this that the evidence for the independence of contractility and conductivity (or excitability) as put forward by Biedermann (1896), Engelmann (1902a; 1902b; 1903a; 1903b) Härtl (1904) and others is inadequate. Arbeiter (1921) has carefully reviewed the early literature on the subject, and, as a result of his own experiments on the frog's heart perfused in the absence of Ca, has concluded that both the disappearance and the reappearance of the mechanical and electrical response in the heart proceed *pari passu*. To demonstrate this he found it necessary to use a mechanical recording device—the "inscripteur à corde"—of great delicacy.[1] With this instrument he noted among other things that it sometimes required as long as 24 hours of perfusion with a Ca-free Ringer solution to produce complete cessation of contraction in the heart. The amplitude of contractions diminished, however, to a tenth or a twentieth of their original size in the course of the first five minutes of perfusion (Cf. Willigen, 1925). Using the same instrument Einthoven and Hugenholtz (1921) demonstrated that when the heart is gradually poisoned with KCl solution, the "sommets des

[1] This instrument is built on the torsion-wire principle with a long style by means of which movement is magnified. The style is placed in front of the slit of the camera.

mécanogrammes et de l'électrogramme deviennent simultanément et régalièrement plus petits" (p. 180), and they conclude that "les deux groupes de phénomènes sont intimement et indissolublement liés entre eux" (p. 184). These authors attribute the result of Mines and other earlier workers to inadequate methods of mechanical recording. Lewis and Drury (1921) and Lewis (1925) concur in the views of Einthoven and Hugenholtz and point out the inadequacy of Engelmann's contention regarding the independence of *excitability*, *conductivity* and *contractility* in the case of heart muscle. They believe it improbable that any one of the properties can exist apart from the other two (see also Schellong, 1924).

In keeping with the results of these investigators, Gasser and Hartree (1924) using a sensitive isometric lever have found that when poisoned with alcohol or hypotonic saline, heat and tension of the excised sartorius diminish together and progress simultaneously to extinction. They also remark that "the present trend of the evidence is in the direction of showing that poisons destroy in muscle the process which is responsible for the manifestations, heat, tension, conduction, and potential change. It is safe to conclude that heat and tension never have been dissociated, and it seems likely that they cannot be" (p. 404).

Beritoff (1924a; 1924b), on the other hand, has, as just mentioned, expressed his belief that the electrical response is to be separated from the mechanical response in severe fatigue, but as the nature of the conditions of mechanical recording is not given fully in this author's paper, the question has been reëxamined in the course of the present investigation.

Method: Intact gastrocnemei of decerebrate frogs have been used with active circulation, and the optical method of recording previously described (Fulton, 1925a; 1925c) and employed in two previous papers (1925d; 1925e) has been used throughout. Fresh preparations were fatigued by prolonged tetanic stimulation first through the nerve and in a few experiments subsequently by direct stimulation. The beginning of the response was recorded, then a "sample" was obtained after about a minute of continuous stimulation, and finally the end of the response was photographed just before the myograph, owing to excessive fatigue, had nearly returned to the base line. Precautions were taken to ensure the absence of local excitation fatigue beneath the stimulating electrodes by moving the electrodes up and down on the nerve *during* stimulation.

Results: Records taken in this way from twenty different preparations have demonstrated clearly and consistently that a muscle which shows any trace of an action current when stimulated through the nerve invariably shows a corresponding trace of mechanical response. Thus in figure 1 a fatigue curve is shown in which a fresh intact muscle was stimulated through its nerve at 30 per second until almost complete extinction of the

electrical responses. The upper exposure shows the beginning "sample" of the responses. Between the end of this exposure and the beginning of the middle record 45 seconds elapsed during which the muscle was stimulated continuously. The lower exposure of this figure was taken 90 seconds after the end of the middle one, as determined with a stop-watch. During the middle exposure the mechanical and electrical responses diminish

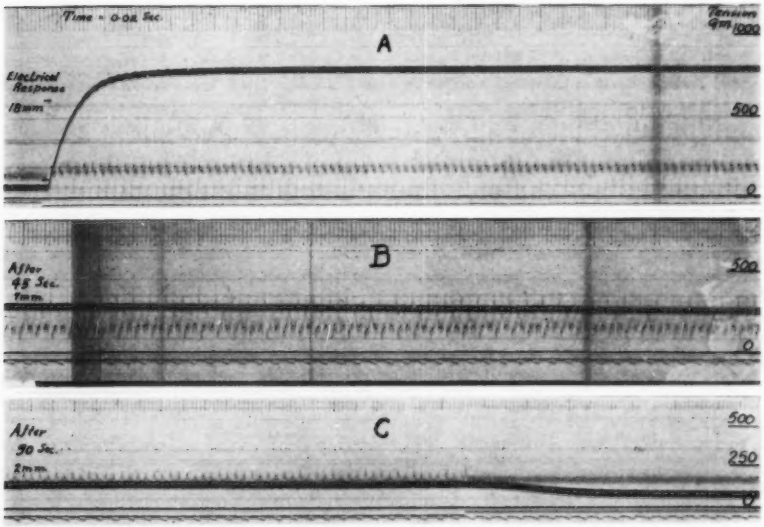

Fig. 1. Fatigue curves of the intact gastrocnemius (14°) stimulated indirectly. The stimulation is commenced in record A, continued in B after an interval of 45 seconds' continuous stimulation, and C is the terminal record of the same tetanus after a further interval of 90 seconds' continuous stimulation. It will be seen that the mechanical and the electrical responses diminish *pari passu*. The very small electrical responses in the last record are represented by a small residual mechanical tension which disappears after cessation of the stimuli. That the diminution in this response was not due to local excitation fatigue was tested in the response (between records B and C) by moving the electrodes up and down on the nerve and this caused no increase in the size of the response.

Note the size of the action currents in A as compared with B and C. Time above — 0.02 second. String tension 7 mm. per m.v. A signal shows the commencement and cessation of stimulation in (A and C).

progressively and proportionally. In the last exposure both the mechanical and the electrical responses are very much diminished. The stimuli were stopped near the end of this record to show that a small increment of mechanical response still remains to account for the small electrical deviations. Such records have always given the same result, and never in my experience has an electrical response existed without

tension. In many records when fatigue was extreme the muscle responded at half the rate of stimulation even though the stimuli were monophasic (50 per sec.)

The same type of curve is obtained with isotonic responses as with isometric, save that fatigue proceeds less rapidly in isotonic than in isometric contractions. This is almost certainly to be attributed to the shorter length of the muscle fibres (1925e) when responding isotonically, for at the shorter length less energy is liberated stimulus for stimulus than when maintained at a greater length as in an isometric response. This is in keeping with a previous observation (1925b) that the greater the initial tension (and length) the more quickly does fatigue show itself in an isometric response by modifying the shape of the terminal "angle" of a tetanus. When stimulated directly, though, owing to "escape" of current, analysis is difficult and less accurate, the same result has been obtained.

In rather more than half the preparations fatigued in this way distinct hyperpnea of the preparation was observed several minutes after the fatiguing stimuli had commenced. As it is often difficult to be certain whether circulation through the recording muscle is vigorous, the occurrence of hyperpnea following stimulation has served as a useful control to blood supply.

Discussion: As fatigue is associated with increase of H-ion concentration within the muscle, the recent results of Drury and Andrus (1924) and of Andrus (1924) are of interest. They found that if the mammalian auricle were perfused with solutions of different H-ion concentration, the rate of rise and decline as well as the total magnitude of the action current became decreased as the perfusate was made more acid. The rate of conduction also diminished with the pH. This suggests that it is the accumulation of H-ions *per se* which causes disappearance of the action current in fatigue (cf. Cobb and Forbes, 1923). Moreover Hartree and Hill (1924) have shown that the lower the pH (the greater the acidity), especially when the H-ion is carried by CO_2, the slower the oxidative processes of recovery.

The polarized interfaces within a muscle responsible for the action current would appear to result from a condition in which certain ions (which, when freely diffused through the sarcoplasm cause a shifting of equilibrium of the "precursor" toward lactic acid formation) are concentrated or in some way prevented from acting upon the energy releasing mechanisms of the muscle cell. When the polarization is temporarily broken down, as by an induced current, a quantum of ions is released and a corresponding quantum of energy is liberated by the break-down of some carbohydrate "precursor" into acid substances. When excessive quantities of acid substances accumulate *within* the cell, polarization of these cell interfaces appears to become less and less complete and the ions which lead to further acid liberation are possibly more freely diffused throughout the cell than normally.

and, owing to mass-action effects, can no longer cause the production of further acid. Such an interpretation (which is really the view set forth by Hartree and Hill (1921, p. 140) modified to stress the importance of the electrical response) provides a reason for the simultaneous disappearance of the electrical response, heat and tension in fatigue. It also stresses the interdependence of excitability (as measured by the electrical response), conductivity (Drury and Andrus), heat production, and contractility,—as Einthoven and Hugenholtz have said, these manifestations are intimately bound up with each other, and it is probably impossible to have one without the other three. All four properties would in fact appear to depend upon *one* fundamental condition, namely, polarization. When the polarized membrane responsible for the action current is broken down permanently as by the action of narcotics, perfusates of high H-ion concentration or (which amounts to the same thing) by severe fatigue, the muscle becomes incapable of contracting, producing heat or conducting excitation.

In view of this marked interdependence, and the fact that the electrical response is regarded as an index of energy liberation (Fulton, 1925e), it follows as a corollary that a supernormal phase in one property, e.g., as contractility, should be accompanied by a corresponding enhanced phase in the other three, e.g., the electrical response. But as a second electrical response never shows a period of "supernormal" size following a previous response, the conclusion reached in a previous paper (1925d) that the supposed supernormal phase of contractility described by Hartree and Hill is due in reality to an inadequate method of analysis, is strengthened.

Furthermore, the distinction made in the case of nerve between conductivity and excitability appear inadequate on many grounds, and Lillie (1914, 1923) among others has emphasized that they cannot be considered apart from each other. It would appear, therefore, that the properties both of muscle and nerve depend for their existence upon one fundamental condition, namely, the existence of a polarized interface endowed with properties of rapid reversibility, and it is this interface which is responsible for the electrical change.

SUMMARY

1. Accurate mechanical records of the prolonged tetanic responses of frog's gastrocnemei (intact) taken simultaneously with electrical responses, have shown that as fatigue progresses the size of the action currents and the degree of tension maintained diminish *pari passu*, and progress simultaneously to extinction.

2. It is pointed out that the evidence for the existence of *conductivity*, *excitability*, and *contractility* as independent and separable properties of muscle is untenable.

3. The view is urged that the properties of excitable tissues in reality

depend for their existence upon one fundamental condition—namely, the existence of the polarized interface responsible for the electrical change.

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